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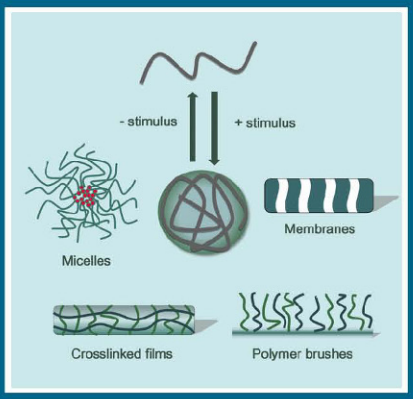

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María Rosa Aguilar and
Julio San Román

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Introduction to smart polymers and their applications

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Abstract: The scientific community tries to mimic nature in the way that living organisms adopt their behavior as a function of environmental conditions to improve survival. In this sense, smart polymers offer materials that respond to numerous stimuli (temperature, pH, electric and magnetic fields, light intensity, biological molecules, etc.), and scientists must devise the best way to apply them in all research areas. This chapter comprehensively summarizes the content of this book which tries to provide a wide overview of *smart polymers* and the most interesting applications developed recently.

Key words: smart polymers, stimuli-responsive polymers, sensitive polymers, applications.

1.1 Introduction

Living systems respond to naturally varying environmental conditions, adapting their structure and functionality to suit these changes by using complex sensing mechanisms, actuating and regulating functions, and feedback control systems. Nature can be considered the best example a scientist can have when developing new materials and applications, and the overall challenge for scientists is to create materials with dynamic and tunable properties, mimicking the active microenvironment that occurs in nature.

Smart polymers or *stimuli-responsive polymers* undergo large reversible changes, either physical or chemical, in their properties as a consequence of small environmental variations. They can respond to a single stimulus or multiple stimuli such as temperature, pH, electric or magnetic field, light intensity, biological molecules, etc., that induce macroscopic responses in the material, such as swelling/collapse or solution-to-gel transitions, depending on the physical state of the chains (Aguilar *et al.*, 2007).

Linear and *solubilized smart macromolecules* will pass from monophasic to biphasic near the transition conditions giving rise to reversible sol–gel hydrogels. *Smart cross-linked networks* undergo chain reorganization at the transition conditions where the network passes from a collapsed to an expanded estate. *Smart surfaces* change their hydrophilicity as a function of a stimulus-providing, responsive interface. All these changes can be used in the design of smart devices for multiple applications, for example, minimally invasive injectable systems (Nguyen and Lee, 2010); pulsatile drug delivery systems (Arora *et al.*, 2011; Tran *et al.*, 2013) or new substrates for cell culture or tissue engineering (Duarte *et al.*, 2011).

Moreover, most polymers can easily be functionalized by pre-polymerization (Guillerm *et al.*, 2012) or post-polymerization (Arnold *et al.*, 2012) methods incorporating functional molecules to the structure, such as biological receptors (Shakya *et al.*, 2010). Therefore, polymer scientists have a wide range of possibilities in terms of polymer chemical structures, polymer architectures and polymer modifications to develop an infinite number of applications for these smart materials (Stuart *et al.*, 2010).

The aim of this book is to guide the reader through the amazing world of smart polymers in order to understand not only the state of the art in this area but also shed some light on future directions in this research field. The first part of the book gives the reader a broad overview of different stimuli-responsive polymers. Temperature, pH, light intensity, magnetic field and enzyme responsive polymers are described. Moreover, due to their actual and future applications, special attention is paid to smart hydrogels, shape memory materials and self-healing polymers.

1.2 Types of smart polymer

Polymers that respond to temperature changes and, more specifically, those that undergo a phase transition in aqueous solution are currently gaining special attention due to their potential applications in the biomaterials field (Bajpai, 2010), architecture (Yang *et al.*, 2013) or water-recovery strategies (Yang *et al.*, 2013) amongst others. *Temperature-sensitive polymers* present low critical solution temperature (LCST) or upper critical solution temperature (UCST) depending on their transition behavior from monophasic to biphasic when temperature is raised or, on the contrary, from biphasic to monophasic when temperature is raised, respectively. LCST polymers have been widely investigated whereas UCST polymers are quite rare. Most common LCST polymers are the poly(*N*-substituted acrylamide), poly(vinyl amide) and poly(oligoethylene glycol (meth)acrylate) families. However, many other polymers can present LCST behavior if the proper hydrophilic–hydrophobic balance is present in the macromolecules. Poly(vinyl ether)s (Aoshima and Kanaoka, 2008), poly(2-oxazoline)s (Guillerm *et al.*,

2012) and poly(phosphoester)s (Turánek *et al.*, 2009) also present temperature-responsive behavior and are reviewed in this book.

pH-sensitive polymers bear weak polyacidic (poly(acrylic acids) or poly(methacrylic acids)) or polybasic (poly(*N*-dimethylaminoethyl methacrylate), poly(*N*-diethylaminoethyl methacrylate), poly(ethyl pyrrolidine methacrylate)) moieties in their structure that protonate or deprotonate as a function of the surrounding pH. Drug delivery systems, gene carriers (Pezzoli and Candiani, 2013) or glucose sensors (Kost and Langer 2012) are three of the multiple applications described for this kind of smart polymer.

Photo-sensitive polymers undergo a reversible or irreversible change in conformation, polarity, amphiphilicity, charge, optical chirality, conjugation, etc., in response to a light stimulus. Reversible chromophores or reversible molecular switches (e.g., azobenzenes, spiropyran, diaryl ethane or coumarin) undergo a reversible isomerization upon light irradiation (Wang and Wang, 2013) while irreversible chromophores are cleaved from the polymer chain upon light exposure (e.g., o-nitrobenzylphotolabile protecting group) or induced reactivity resulting in the coupling of two species (e.g., 2-naphthoquinone-3-methides). Both molecular switches and irreversible chromophores have been applied in multiple applications such as drug delivery systems, functional micropatterns, responsive hydrogels, photodegradable materials or photoswitchable liquid crystalline elastomers for remote actuation (Ohm *et al.*, 2010).

Polymer hydrogels play a key role in the development of new biomaterials as their high level of hydration and 3D structure resemble natural tissue. However, despite the superior performance of hydrogels, they present several limitations mainly due to their poor controllability, actuation and response polymers. Several advances have been made in this sense by the use of smart polymers in the preparation of hydrogels (Ravichandran *et al.*, 2012). For example, *magnetically responsive polymer gels* and *elastomers* are composites based on magnetic nanoparticles dispersed in a high elastic polymeric matrix. Magnetic field quickly deforms the polymer matrix with no noise, heat evolution or exhaustion which makes these materials ideal for the preparation of sensors, micromachines, energy transducing devices, controlled delivery systems or even artificial muscles (Li *et al.*, 2013). One of the limiting steps in the development of these materials has been the precise coupling of magnetic nanoparticles to the gel; however, this problem has been overcome when magnetic nanoparticles form the cross-linking nodes of the hydrogel (Ilg, 2013).

Macroscopic transitions can also be triggered by 'biology-to-material' interactions in the so-called *biointeractive polymers*. These materials incorporate receptors for biomolecules which, when stimulated, cause localized or bulk modifications in the material properties. Those polymers that respond to selective enzyme catalysis are known as *enzyme responsive*

polymers. These materials represent an important advance in the integration of artificial materials with biological entities as they link together the polymer properties with specific biological processes controlled naturally by either regulating enzyme expression levels or availability of cofactors (Hu *et al.*, 2012b). Enzyme responsive polymers can also display reversible and dynamic responses to a stimulus, thus being of great interest in the formulation of new biomaterials such as cell supports, injectable scaffolds or drug delivery systems (de la Rica *et al.*, 2012).

Shape memory polymers represent one of the most active areas in material science due to their easier processability and lower cost when compared with shape memory metals or ceramics. This kind of smart polymers have the ability to recover their (permanent) predefined shape when stimulated by an external stimulus. A stable network and a reversible switching transition of the polymer are the two pre-requisites for shape memory effect. The stable network is responsible for the original shape, and reversible switching transition fixes the temporary shape, which can be crystallization/melting transition, liquid crystal anisotropic/isotropic transition, reversible molecule cross-linking (such as photodimerization, Diels – Alder reaction, oxidation/redox reaction of mercapto groups), and supramolecular association/disassociation (such as hydrogen bonding, self-assembly metal–ligand coordination and self-assembly of β-cyclodextrin). In addition to the reversible switches mentioned, other stimuli that change chain mobility can also trigger shape memory effect, such as light, pH, moisture, electric field, magnetic field, pressure, etc. (Pretsch, 2010). Shape memory polymers allow large, recoverable strains; however, they normally present poor mechanical properties and do not support great shape-recovery stresses. As a result, great efforts are being made in the development of shape memory composites with reinforced properties. Shape memory polymers present numerous actual and potential applications in medicine, aerospace, textiles, engineering, microfluidics, lithography and household products (Meng and Li, 2013).

Self-healing or restoration of lost functionalities without external help is a dream come true with self-healing polymers (Aïssa *et al.*, 2012). Healing mechanisms can be extrinsic (the healing compound is isolated from the polymer matrix in capsules, fibers or nanocarriers) or intrinsic (the polymer chains temporarily increase mobility and flow to the damaged area) (Billiet *et al.*, 2013) and are responsible for restoration of properties such as structural integrity (White *et al.*, 2001), surface aesthetics (Yao *et al.*, 2011), electrical conductivity (Tee *et al.*, 2012), hydrophobicity and hydrophilicity (Ionov and Synytska, 2012), mechanical properties (Jones *et al.*, 2013), etc.

1.3 Applications of smart polymers

The second part of the book comprises relevant applications of smart polymers and their future trends according to the opinion of well-known

researchers in the field. Most of the important developments were registered in the biomedical field and use smart polymers in the development of new therapies for the treatment of several diseases or sophisticated medical devices that react to the environment of the surrounding tissues (pH, temperature, enzymes, analyte concentration, etc.) or external stimuli (light, magnetic radiation, etc.).

Responsive polymeric substrates or *instructive substrates* regulate cell behavior in response to external factors and are of significant importance in tissue engineering applications (Pérez *et al.*, 2013). Cell behavior (adhesion, migration and proliferation) is conditioned to substrate surface properties (Alves *et al.*, 2010). Tunable surface properties such as stiffness and wettability, surface functionalization with bioactive molecules or the design of 3D patterns at the micro- or nanoscale in hydrogels are interesting strategies actually being developed in order to obtain specific cell responses to smart surfaces for tissue engineering applications (e.g., cell sheet engineering (Haraguchi *et al.*, 2012), smart biomineralization (Huang *et al.*, 2008), heart valve and vascular graft tissue engineering (Fioretta *et al.*, 2012)), drug delivery (Moroni *et al.*, 2008), cell recruitment (Custódio *et al.*, 2012) or the development of new and more effective medical devices.

Temperature-sensitive polymers and more specifically *shape memory polymers* have been used in the preparation of *minimally invasive surgery* medical devices (Yakacki and Gall, 2010). The unique properties of these materials allow the introduction of the medical device in a compressed form followed by expansion once located in the desired place by minimally invasive surgery procedures. One of the most relevant applications using this kind of polymer is the development of stents for either vascular or urologic procedures. Polymeric stents are considered a promising option when compared to the conventional metallic stents due not only to their mechanical properties but also to the possibility of incorporating a drug to be eluted in the functional place (e.g., to reduce restenosis and/or thrombosis after implantation in vascular stents or to minimize infections in urinary stents (Xue *et al.*, 2012)).

Smart polymers have played a key role in the fabrication of new medical devices for cancer diagnosis and therapy. In this sense, magnetic nanoparticles have been used in the development of hyperthermia treatments, magnetic separation, immunoassay, cellular labeling and magnetic resonance imaging diagnosis (Karimi *et al.*, 2013). Biosensors based on smart polymers have been used in clinical diagnosis and forensic analysis because alterations in the concentration of certain analytes (e.g., glucose in diabetes (Thammakhet *et al.*, 2011)) or in physical variables such as temperature or pH (e.g., pH sensor for the quantification of partial pressure of CO_2 in the stomach for the diagnosis of gastrointestinal ischemia (Herber *et al.*, 2005)) occur in several diseases. Biosensors and actuators have been also

combined in medical devices, for example, a glucose-sensing and insulin delivery medical device (Brahim *et al.*, 2002) or cochlear implant (Laursen, 2006). Microfluidics-based medical devices or *Lab on a Chip* also combine biosensors to detect systemic levels of certain analytes and actuators to release bioactive components in response to excessive or insufficient concentrations of these analytes (Do *et al.*, 2008).

Smart polymer nanocarriers for drug delivery applications play an important role in the development of highly active and selective treatments, as they permit a controlled delivery of the drug in the right place at the right time (Fleige *et al.*, 2012). Better knowledge of the molecular biology and synthesis of new polymers with stimulus-sensitive moieties gave rise to more effective, specifically localized, action and personalized therapies. This is the case for human neutrophil elastase degradable links that are specifically degraded at inflammation sites where neutrophils play their action (Aimetti *et al.*, 2009; Fleige *et al.*, 2012) and also for cathepsin B-sensitive polyglutamates that are better degraded in women than in men, due to the activity of lysosomal cysteine protease cathepsin B enzyme closely correlated with estrogen levels (Lammers *et al.*, 2012).

Smart polymers have also been used for bioseparation and other biotechnological applications such as purification techniques (Galaev and Mattiasson, 2007). New smart polymers have brought about progress in affinity precipitation (Gautam *et al.*, 2012), aqueous polymer two phase partitioning (Qu *et al.*, 2010), controlled permeation membranes (Wang and Chen, 2007) and thermosensitive chromatography (Kanno *et al.*, 2011).

Textiles have also experienced great improvements through the incorporation of different kinds of smart polymers to their formulations. Temperature, pH, moisture or light were responsible for the variable aesthetic appeal, smart controlled drug release, wound monitoring or smart wetting properties of new textiles. Moreover, protection against extreme variations in environmental conditions or textiles with medical properties were also achieved with the use of smart polymers (Hu *et al.*, 2012a).

Another sector clearly benefiting from smart polymers is the food industry as smart micro- or nanoparticles have been used for incorporating active ingredients (e.g., ascorbic acid (Devi and Kakati, 2013) or olive oil (Devi *et al.*, 2012)) in food or antimicrobial polymers such as chitosan, which have been used to fabricate edible coatings (Fernandez-Saiz *et al.*, 2010).

Information and communication technologies and more specifically data storage devices have improved amazingly in recent years due to the fabrication of new smart materials. In this way, volume holographic storage will give rise to the next generation of data storage devices, due to their much higher storage capacity and much higher transfer rate when compared

with actual 2D optical discs (Garan, 2013). In this sense, azobenzene chromophores stand by their capacity to induce optical anisotropy when incorporated in photoaddressable polymeric materials (Shishido, 2010).

1.4 Conclusion

Multidisciplinary research involving scientists of very different disciplines will be required to make future advances in smart polymers and their applications. Organic chemists, polymer chemists, material engineers, physics, biologists, pharmacists and medical doctors will have to work together in a very close and fluid manner to respond to the necessities of society and developing new materials that will improve the quality of life, not only in the medical field, but also in the areas of architecture, textiles, food, data storage, etc.

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1.6 References

Aguilar, M. R., Elvira, C., Gallardo, A., Vázquez, B. and San Román, J. (2007) Smart polymers and their applications as biomaterials. In: Ashammakhi, N., Reis, R. L. and Chiellini, E. (eds.) *Topics in Tissue Engineering, e-book*. Expertissues, Chapter 6, 27 pp.

Aimetti, A. A., Machen, A. J. and Anseth, K. S. (2009) Poly(ethylene glycol) hydrogels formed by thiol-ene photopolymerization for enzyme-responsive protein delivery. *Biomaterials*, **30**, 6048–6054.

Aïssa, B., Therriault, D., Haddad, E. and Jamroz, W. (2012) Self-healing materials systems: Overview of major approaches and recent developed technologies. *Advances in Materials Science and Engineering*, **2012**, 17 pp., Article ID 854203.

Alves, N. M., Pashkuleva, I., Reis, R. L. and Mano, J. F. (2010) Controlling cell behavior through the design of polymer surfaces. *Small*, **6**, 2208–2220.

Aoshima, S. and Kanaoka, S. (2008) Synthesis of stimuli-responsive polymers by living polymerization: Poly(N-isopropylacrylamide) and poly(vinyl ether)s. *Advances in Polymer Science,* **210**, 169–208.

Arnold, R. M., Huddleston, N. E. and Locklin, J. (2012) Utilizing click chemistry to design functional interfaces through post-polymerization modification. *Journal of Materials Chemistry*, **22**, 19357–19365.

Arora, G., Singh, I., Nagpal, M. and Arora, S. (2011) Recent advances in stimuli induced pulsatile drug delivery system: A review. *Research Journal of Pharmacy and Technology*, **4**, 691–703.

Bajpai, A., Shukla, S., Saini, R. and Tiwari, A. (eds.) (2010) Temperature sensitive release systems. In: *Stimuli Responsive Drug Delivery Systems: From Introduction to Application* UK, Smithers Rapra Technologies.

Billiet, S., Hillewaere, X. K. D., Teixeira, R. F. A. and Du Prez, F. E. (2013) Chemistry of crosslinking processes for self-healing polymers. *Macromolecular Rapid Communications*, **34**, 290–309.

Brahim, S., Narinesingh, D. and Guiseppi-Elie, A. (2002) Bio-smart hydrogels: Co-joined molecular recognition and signal transduction in biosensor fabrication and drug delivery. *Biosensors and Bioelectronics*, **17**, 973–981.

Custódio, C. A., Frias, A. M., del Campo, A., Reis, R. L. and Mano, J. F. (2012) Selective cell recruitment and spatially controlled cell attachment on instructive chitosan surfaces functionalized with antibodies. *Biointerphases*, **7**, 1–9.

de la Rica, R., Aili, D. and Stevens, M. M. (2012) Enzyme-responsive nanoparticles for drug release and diagnostics. *Advanced Drug Delivery Reviews*, **64**, 967–978.

Devi, N. and Kakati, D. K. (2013) Smart porous microparticles based on gelatin/sodium alginate polyelectrolyte complex. *Journal of Food Engineering*, **117**, 193–204.

Devi, N., Hazarika, D., Deka, C. and Kakati, D. K. (2012) Study of complex coacervation of gelatin a and sodium alginate for microencapsulation of olive oil. *Journal of Macromolecular Science, Part A: Pure and Applied Chemistry*, **49**, 936–945.

Do, J., Lee, S., Han, J., Kai, J., Hong, C. C., Gao, C., Nevin, J. H., Beaucage, G. and Ahn, C. H. (2008) Development of functional lab-on-a-chip on polymer for point-of-care testing of metabolic parameters. *Lab on a Chip – Miniaturisation for Chemistry and Biology*, **8**, 2113–2120.

Duarte, A. R. C., Mano, J. F. and Reis, R. L. (2011) Thermosensitive polymeric matrices for three-dimensional cell culture strategies. *Acta Biomaterialia*, **7**, 526–529.

Fernandez-Saiz, P., Ocio, M. J. and Lagaron, J. M. (2010) The use of chitosan in antimicrobial films for food protection. *CAB Reviews: Perspectives in Agriculture, Veterinary Science*, *Nutrition and Natural Resources*, **5**, 1–11.

Fioretta, E. S., Fledderus, J. O., Burakowska-Meise, E. A., Baaijens, F. P. T., Verhaar, M. C. and Bouten, C. V. C. (2012) Polymer-based scaffold designs for in situ vascular tissue engineering: controlling recruitment and differentiation behavior of endothelial colony forming cells. *Macromolecular Bioscience*, **12**, 577–590.

Fleige, E., Quadir, M. A. and Haag, R. (2012) Stimuli-responsive polymeric nanocarriers for the controlled transport of active compounds: Concepts and applications. *Advanced Drug Delivery Reviews*, **64**, 866–884.

Galaev, I. and Mattiasson, B. (eds) (2007) *Smart Polymers. Applications in Biotechnology and Biomedicine*, Boca Ratón, USA, CRC Press, Taylor and Francis Group.

Garan, S. S. (2013) Holographic imaging: Information recording and retrieval. *Journal of the Indian Institute of Science*, **93**, 35–45.

Gautam, S., Dubey, P., Varadarajan, R. and Gupta, M. N. (2012) Role of smart polymers in protein purification and refolding. *Bioengineered Bugs*, **3**, 286–288

Guillerm, B., Monge, S., Lapinte, V. and Robin, J. J. (2012) How to modulate the chemical structure of polyoxazolines by appropriate functionalization. *Macromolecular Rapid Communications*, **33**, 1600–1612.

Haraguchi, Y., Shimizu, T., Yamato, M. and Okano, T. (2012) Scaffold-free tissue engineering using cell sheet technology. *RSC Advances*, **2**, 2184–2190.

Herber, S., Bomer, J., Olthuis, W., Bergveld, P. and van den Berg, A. (2005) A miniaturized carbon dioxide gas sensor based on sensing of pH-sensitive hydrogel swelling with a pressure sensor. *Biomedical Microdevices*, **7**, 197–204.

Hu, J., Meng, H., Li, G. and Ibekwe, S. I. (2012a) A review of stimuli-responsive polymers for smart textile applications. *Smart Materials and Structures*, **21**, 053001 (23pp.).

Hu, J., Zhang, G. and Liu, S. (2012b) Enzyme-responsive polymeric assemblies, nanoparticles and hydrogels. *Chemical Society Reviews*, **41**, 5933–5949.

Huang, Z., Sargeant, T. D., Hulvat, J. F., Mata, A., Bringas Jr, P., Koh, C. Y., Stupp, S. I. and Snead, M. L. (2008) Bioactive nanofibers instruct cells to proliferate and differentiate during enamel regeneration. *Journal of Bone and Mineral Research*, **23**, 1995–2006.

Ilg, P. (2013) Stimuli-responsive hydrogels cross-linked by magnetic nanoparticles. *Soft Matter*, **9**, 3465–3468.

Ionov, L. and Synytska, A. (2012) Self-healing superhydrophobic materials. *Physical Chemistry Chemical Physics*, **14**, 10497–10502.

Jones, A. R., Blaiszik, B. J., White, S. R. and Sottos, N. R. (2013) Full recovery of fiber/matrix interfacial bond strength using a microencapsulated solvent-based healing system. *Composites Science and Technology*, **79**, 1–7.

Kanno, S., Watanabe, K., Yamagishi, I., Hirano, S., Minakata, K., Gonmori, K. and Suzuki, O. (2011) Simultaneous analysis of cardiac glycosides in blood and urine by thermoresponsive LC-MS-MS. *Analytical and Bioanalytical Chemistry*, **399**, 1141–1149.

Karimi, Z., Karimi, L. and Shokrollahi, H. (2013) Nano-magnetic particles used in biomedicine: Core and coating materials. *Materials Science and Engineering C*, **33**, 2465–2475.

Kost, J. and Langer, R. (2012) Responsive polymeric delivery systems. *Advanced Drug Delivery Reviews*, **64**, 327–341.

Lammers, T., Kiessling, F., Hennink, W. E. and Storm, G. (2012) Drug targeting to tumors: Principles, pitfalls and (pre-) clinical progress. *Journal of Controlled Release*, **161**, 175–187.

Laursen, W. (2006) Breaking the sound barrier [cochlear implants]. *Engineering and Technology*, **1**, 38–41.

Li, Y., Huang, G., Zhang, X., Li, B., Chen, Y., Lu, T., Lu, T. J. and Xu, F. (2013) Magnetic hydrogels and their potential biomedical applications. *Advanced Functional Materials*, **23**, 660–672.

Meng, H. and Li, G. (2013) A review of stimuli-responsive shape memory polymer composites. *Polymer* (United Kingdom), **54**, 2199–2221.

Moroni, L., De Wijn, J. R. and Van Blitterswijk, C. A. (2008) Integrating novel technologies to fabricate smart scaffolds. *Journal of Biomaterials Science, Polymer Edition*, **19**, 543–572.

Nguyen, M. K. and Lee, D. S. (2010) Injectable biodegradable hydrogels. *Macromolecular Bioscience*, **10**, 563–579.

Ohm, C., Brehmer, M. and Zentel, R. (2010) Liquid crystalline elastomers as actuators and sensors. *Advanced Materials*, **22**, 3366–3387.

Pérez, R. A., Won, J. E., Knowles, J. C. and Kim, H. W. (2013) Naturally and synthetic smart composite biomaterials for tissue regeneration. *Advanced Drug Delivery Reviews*, **65**, 471–496.

Pezzoli, D. and Candiani, G. (2013) Non-viral gene delivery strategies for gene therapy: A 'ménage à trois' among nucleic acids, materials, and the biological environment: Stimuli-responsive gene delivery vectors. *Journal of Nanoparticle Research*, **15**, Article number 1523.

Pretsch, T. (2010) Review on the functional determinants and durability of shape memory polymers. *Polymers*, **2**, 120–158.

Qu, F., Lü, F. and Zhang, H. (2010) Smart polymer based aqueous two-phase systems applied in bio-molecule separation and purification. *Progress in Chemistry*, **22**, 125–132.

Ravichandran, R., Sundarrajan, S., Venugopal, J. R., Mukherjee, S. and Ramakrishna, S. (2012) Advances in polymeric systems for tissue engineering and biomedical applications. *Macromolecular Bioscience*, **12**, 286–311.

Shakya, A. K., Sami, H., Srivastava, A. and Kumar, A. (2010) Stability of responsive polymer-protein bioconjugates. *Progress in Polymer Science (Oxford)*, **35**, 459–486.

Shishido, A. (2010) Rewritable holograms based on azobenzene-containing liquid-crystalline polymers. *Polymer Journal*, **42**, 525–533.

Stuart, M. A. C., Huck, W. T. S., Genzer, J., Müller, M., Ober, C., Stamm, M., Sukhorukov, G. B., Szleifer, I., Tsukruk, V. V., Urban, M., Winnik, F., Zauscher, S., Luzinov, I. and Minko, S. (2010) Emerging applications of stimuli-responsive polymer materials. *Nature Materials*, **9**, 101–113.

Tee, B. C. K., Wang, C., Allen, R. and Bao, Z. (2012) An electrically and mechanically self-healing composite with pressure- and flexion-sensitive properties for electronic skin applications. *Nature Nanotechnology*, **7**, 825–832.

Thammakhet, C., Thavarungkul, P. and Kanatharana, P. (2011) Development of an on-column affinity smart polymer gel glucose sensor. *Analytica Chimica Acta*, **695**, 105–112.

Tran, P. H. L., Tran, T. T. D., Vo, V. T. and Lee, B. J. (2013) pH-sensitive polymeric systems for controlling drug release in nocturnal asthma treatment, 4th International Conference on the Development of Biomedical Engineering in Vietnam, 8–12 January 2012 Ho Chi Minh City, Vietnam. Springer, 304–308.

Turánek, J., Wang, X. F., Knötigová, P., Koudelka, Å., Dong, L. F., Vrublová, E., Mahdavian, E., Procházka, L., Sangsura, S., Vacek, A., Salvatore, B. A. and Neuzil, J. (2009) Liposomal formulation of a-tocopheryl maleamide: In vitro and in vivo toxicological profile and anticancer effect against spontaneous breast carcinomas in mice. *Toxicology and Applied Pharmacology*, **237**, 249–257.

Wang, D. and Wang, X. (2013) Amphiphilic azo polymers: Molecular engineering, self-assembly and photoresponsive properties. *Progress in Polymer Science*, **38**, 271–301.

Wang, W. and Chen, L. (2007) 'Smart' membrane materials: Preparation and characterization of PVDF-g-PNIPAAm graft copolymer. *Journal of Applied Polymer Science*, **104**, 1482–1486.

White, S. R., Sottos, N. R., Geubelle, P. H., Moore, J. S., Kessler, M. R., Sriram, S. R., Brown, E. N. and Viswanathan, S. (2001) Autonomic healing of polymer composites. *Nature*, **409**, 794–797.

Xue, L., Dai, S. and Li, Z. (2012) Synthesis and characterization of elastic star shape-memory polymers as self-expandable drug-eluting stents. *Journal of Materials Chemistry*, **22**, 7403–7411.

Yakacki, C. M. and Gall, K. (2010) Shape-memory polymers for biomedical applications. *Advances in Polymer Science,* **226,** 147–175.

Yang, H., Zhu, H., Hendrix, M. M. R. M., Lousberg, N. J. H. G. M., De With, G., Esteves, A. C. C. and Xin, J. H. (2013) Temperature-triggered collection and release of water from fogs by a sponge-like cotton fabric. *Advanced Materials*, **25**, 1150–1154.

Yao, L., Yuan, Y. C., Rong, M. Z. and Zhang, M. Q. (2011) Self-healing linear polymers based on RAFT polymerization. *Polymer*, **52**, 3137–3145.

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Part I

Types of smart polymer

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2

Temperature-responsive polymers: properties, synthesis and applications

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Abstract: This chapter describes polymers that undergo a temperature-induced phase transition in aqueous solution providing an important basis for smart materials. Different types of temperature-responsive polymers, including shape-memory materials, liquid crystalline materials and responsive polymer solutions are briefly introduced. Subsequently this chapter will focus on thermoresponsive polymer solutions. At first, the basic principles of the upper and lower critical temperature polymer phase transitions will be discussed, followed by an overview and discussion of important aspects of various key types of such temperature-responsive polymers. Finally, selected potential applications of thermoresponsive polymer solutions will be described.

Key words: smart material, responsive polymer, lower critical solution temperature (LCST), upper critical solution temperature (UCST), phase transition.

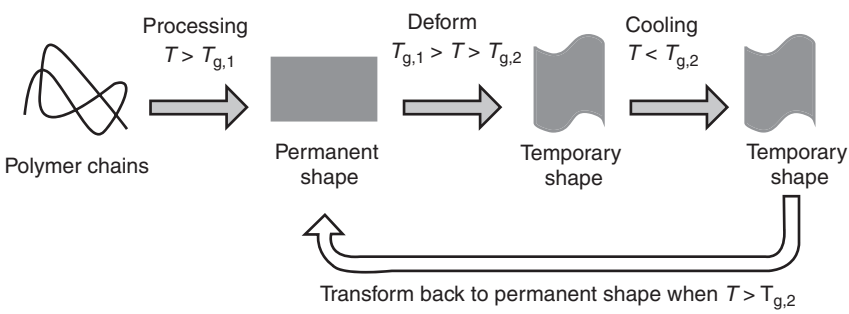
2.1 Introduction

Smart materials that respond with a property change to a variation in the environmental conditions are an attractive class of materials for advanced applications. Responsive and adaptive materials are also omnipresent in natural systems. Examples include the focusing of the eye, opening and closing of pores, and wound healing (Stryer, 1999). The majority of such natural responsive and adaptive processes are driven by conformational changes and/or aggregation of proteins, which can be regarded as nature's smart polymers. Similarly, responsive synthetic polymers are attractive building blocks for the development of artificial smart materials. A wide variety of responsive polymer materials have been reported that respond to various external parameters, such as temperature, pH, mechanical stress and even certain molecules, including CO_2 and sugars (see Roy *et al.*, 2010 for a recent review). The response of the polymer can also be manifold, such as a change in shape, color or solubility.

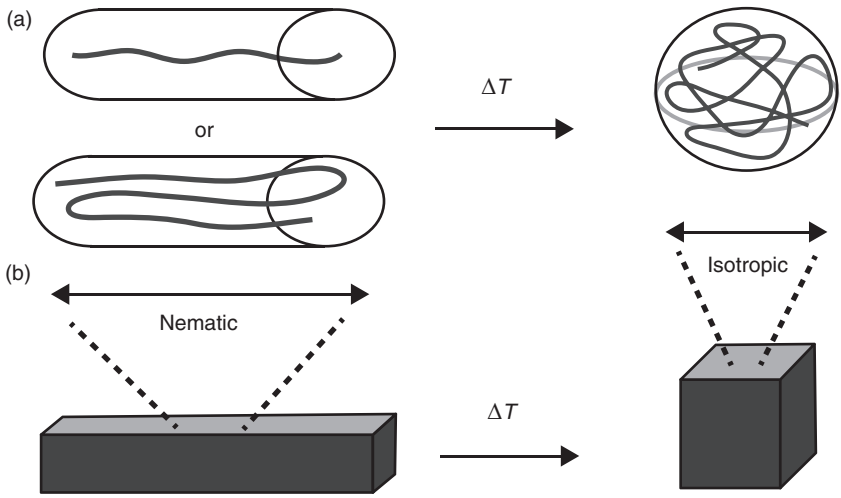
Temperature-responsive polymers are especially interesting since variations in temperature can be applied externally in a non-invasive manner. Furthermore, spontaneous temperature fluctuations also occur in nature, for example, during day and night cycles as well as the increased temperature of inflamed tissue. Different working mechanisms can be exploited for the development of temperature-responsive polymers as will be briefly outlined in this introductory paragraph. The three main classes of temperature-responsive polymers are:

1. shape-memory materials;
2. liquid crystalline materials;
3. responsive polymer solutions.

Shape-memory materials are thermoplastic elastomers consisting of a hard phase with a high glass transition temperature (T_g; $T_{g,1}$ in Fig. 2.1) and a second switching phase with intermediate $T_{g,2}$ or melting temperature that enables the temperature-responsive behavior (Lendlein and Kelch, 2002; Liu *et al.*, 2007). Such shape-memory materials can be deformed in any shape when heating above the highest T_g resulting in the permanent shape. When these materials are subsequently deformed in between the two transition temperatures a temporary shape can be induced, which can be 'frozen' in by cooling the deformed state below the switching temperature. This shape-memory material will transform back to the permanent shape when heated above the switching temperature (Fig. 2.1). As such, these materials are thermoresponsive, but they have to be 'reprogrammed' after each switching cycle. By introducing multiple intermediate temperature transitions, the number of programmable shape changes can be increased and a recent example demonstrated four independent states in a shape-memory material having one broad T_g (Xie, 2010).



2.1 Schematic representation of the thermoresponsive behavior of a shape-memory polymer. $T_{g,1}$ represents the T_g of the hard phase and $T_{g,2}$ represents the T_g of the switching phase.



2.2 (a) Change in conformations of main-chain LC polymers from the extended nematic phase to a collapsed isotropic phase upon heating. (b) Corresponding macroscopic shape change during this nematic–isotropic phase transition.

Liquid crystalline polymers have a liquid crystalline phase in addition to the glassy state and the isotropic rubbery phase (Donald *et al*., 2005; Weiss and Ober, 1990). This liquid crystalline phase has a certain anisotropic order of the mesogens present in the polymer. It has been reported that polymers with main-chain nematic liquid crystalline blocks have an elongated main-chain in the liquid crystalline phase that contracts to a random coil state when heated to the isotropic phase, which is a fully reversible polymer phase transition that has been utilized as the main switching mechanism for developing artificial muscles (Fig. 2.2) (Li and Keller, 2006). Up to 40% contraction has been demonstrated for such materials upon heating. Polymeric networks with side-chain mesogens have also been developed having a chiral nematic liquid crystalline phase as is also used in liquid crystal display (LCD) screens. Such side-chain liquid crystalline polymer networks have been utilized in, for example, the development of thermochromic materials (Sage, 2011).

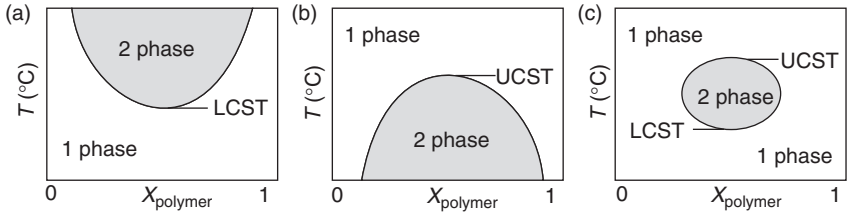
The third and most widely studied type of thermoresponsive polymers are polymers that undergo a solution liquid–liquid phase transition in response to variation of the temperature, that is, phase separation occurs from a homogeneous solution into a concentrated polymer phase and a diluted polymer phase. This phase transition is often accompanied by a transition from a clear solution to a cloudy solution, also known as the cloud point temperature (T_{CP}), for low concentration polymer solutions. This clouding is due to

the formation of droplets of the high concentration polymer phase in combination with the difference in refractive index between the two phases. When the phase separation occurs at an elevated temperature, this is referred to as lower critical solution temperature (LCST) transition while the reversed phase behavior is known as upper critical solution temperature (UCST) transition. Early examples of LCST and UCST behavior of polymers were reported in organic solvents, such as the UCST of poly(styrene) in cyclohexane (Schultz and Flory, 1952) and the LCST of poly(methyl methacrylate) (PMMA) in 2-propanone (Cowie and McEwen, 1976).

Most interesting, however, are thermoresponsive polymer phase transitions in aqueous solutions since this phenomenon provides high potential for biomedical applications, such as drug delivery and switchable synthetic cell culture surfaces (de las Heras Alarcón *et al.*, 2005; Schmaljohann, 2006; Ward and Theoni, 2011). The remainder of this chapter will focus on such temperature-responsive polymers in aqueous solution, by discussing basic principles (Section 2.2) and key types of temperature-responsive polymers (Section 2.3) as well as selected applications (Section 2.4).

2.2 Basic principles of temperature-responsive polymers in aqueous solution

The different types of polymer phase transitions that can occur in aqueous solutions of homopolymers are schematically depicted in Fig. 2.3, namely LCST transition, UCST transition and closed loop coexistence of LCST and UCST transitions. These schematically drawn binodal or coexistence curves represent the equilibrium concentration of the two phases in the phase separated state. The LCST is defined as the lowest temperature of this binodal curve (Fig. 2.3a) while the UCST is defined as the highest temperature of this binodal curve (Fig. 2.3b). Closed loop coexistence has also been reported for a small number of polymers that have coinciding LCST and UCST phase behavior (Fig. 2.3c). The most prominent example of a polymer with such closed loop coexistence in water is poly(ethylene glycol); albeit both LCST and UCST transitions only occur when heated beyond the boiling point of water in closed vessels (Saeki *et al.*, 1976). Other polymers exhibiting closed loop coexistence phase behavior include partially acetylated poly(vinyl alcohol) (Nord *et al.*, 1951) and poly(hydroxyethyl methacrylate) (Longenecker *et al.*, 2011). Besides these three types of polymer phase diagrams, there are also a few examples of polymers that show a low temperature UCST transition and a high temperature LCST transition (not shown in Fig. 2.3), including poly(vinyl methyl ether) (van Assche *et al.*, 2011) and mixtures of poly(dimethylaminoethyl methacrylate) with a trivalent $[Co(CN)_6]^{3-}$ anion (Plamper *et al.*, 2007).

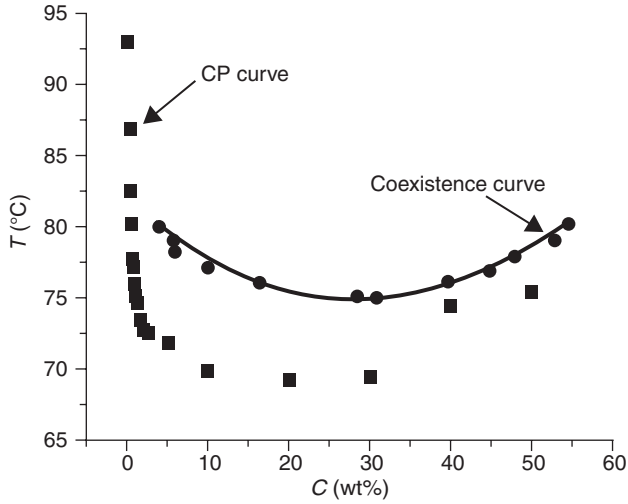


2.3 Schematic representation of the polymer phase diagrams (binodal or coexistence curves) for polymers exhibiting LCST behavior (a), UCST behavior (b) and closed loop coexistence (c).

The majority of recent reports on thermoresponsive polymers evaluate the phase transition temperature at a certain polymer concentration by turbidity measurements, that is, light scattering of a polymer solution at 500–700 nm as function of temperature. It is important to note that the transition temperature thus obtained is the T_{CP}, whereby concentration should be specified, and not the LCST. Furthermore, one should be aware of potential kinetic effects during turbidimetry resulting in a kinetic phase diagram at a certain heating or cooling rate rather than an equilibrium phase diagram, as we recently reported for the LCST transition of a comb-shaped poly[oligo(2-ethyl-2-oxazoline)methacrylate] (Fig. 2.4) (Weber *et al.*, 2013). The basic underlying mechanisms for LCST and UCST polymer phase transitions in aqueous solutions will be discussed in the following paragraphs.

2.2.1 Polymers with lower critical solution temperature (LCST) behavior

Polymers that undergo an LCST transition in water are soluble in water at low temperatures and phase separate upon increasing the temperature. From a thermodynamic point of view this means that the Gibbs free energy ($\Delta G = \Delta H - T\Delta S$) of dissolving the polymer in water is negative at lower temperatures and that it becomes positive upon increasing the temperature. Such behavior is possible only if the enthalpy of dissolution (ΔH) is negative, that is, favorable hydrogen bonding between water molecules and the polymer chains (hydration) and the entropy contribution (ΔS) is negative too; that is, water loses entropy when it is hydrated to the polymer chains. Upon increasing the temperature, the enthalpic hydrogen bonding interaction will become less, but more importantly the entropy term ($-T\Delta S$) will become dominant leading to a positive Gibbs free energy of mixing, thus leading to phase separation. In other words, at elevated temperatures the hydrated water molecules will go back to the bulk water leaving behind partially dehydrated polymer chains that will collapse and aggregate into



2.4 Cloud point temperature (CP, ■; determined at 1 K min^{-1}) and coexistence (●; determined by refractive index of the low and high polymer concentration phases after 24 h equilibration) curves obtained for a binary mixture of a comb-shaped poly[oligo(2-ethyl-2-oxazoline) methacrylate] in water. The coexistence curve is fitted to guide the eye (Weber *et al.*, 2013). (*Source*: Reprinted with permission from Wiley.)

a polymer rich phase. The LCST transition is a fully reversible cooperative (de)hydration process providing access to sharp reversible temperature induced polymer phase transitions. In theory, all water-soluble polymers should undergo such an LCST phase transition in water, but in practice the phase transition cannot always be observed, especially not in water at ambient pressure, and superheating of the solution might be required as is the case for poly(ethylene glycol) (Saeki *et al.*, 1976).

In general, the LCST or T_{CP} strongly depends on the polymer structure. As a general rule of thumb, it can be stated that better hydrated polymers have a higher T_{CP} than less hydrated polymers. As such, increasing the molecular weight of a polymer, which decreases its hydration due to enhanced polymer–polymer interactions, will lead to a lower T_{CP}. Furthermore, introducing hydrophilic end-groups or more hydrophilic (co)monomers will also increase the T_{CP}.

2.2.2 Polymers with upper critical solution temperature (UCST) behavior

The thermodynamic effects of polymer hydration upon dissolution are the same for all polymers and, thus, both ΔH and ΔS for hydration are negative,

in principle leading to LCST behavior, *vide supra* (Section 2.2.1). However, to obtain and understand UCST behavior, another enthalpic term has to be introduced in the Gibbs free energy equation, namely ΔH for supramolecular association of the polymer chains. If the polymer chains have strong associative interactions that have to be broken upon polymer dissolution, this can render the polymer insoluble if this loss in energy is larger than the gain in energy upon dissolution. However, the supramolecular associative interaction strength decreases with increasing temperature leading to the hydration term becoming dominant and, thus, leading to dissolution of the polymer. The polymer, however, should itself be very hydrophilic to avoid that its potential LCST transition is lower than the UCST transition, which would lead to complete insolubility. An important difference between LCST and UCST transitions is that the LCST transition is a cooperative entropy driven process while the UCST transition is an enthalpic process leading to a much shallower transition as has been demonstrated by the incorporation of pyrene as probe for the phase transition (Pietsch *et al.*, 2010a, b).

As the UCST phase transition in water is based on associative interactions, the dependency of the transition temperature will be directly correlated to the strength of the supramolecular interactions. This may lead to counterintuitive effects, such as increased transition temperatures upon incorporation of hydrophobic side chains. Even though hydrophobic side chains decrease the solubility of a polymer and would lower a LCST transition, they can also create a hydrophobic environment for associative hydrogen bonding interactions. This hydrophobic environment enhances the hydrogen bonding strength leading to a higher UCST transition temperature (Seuring and Agarwal, 2012a).

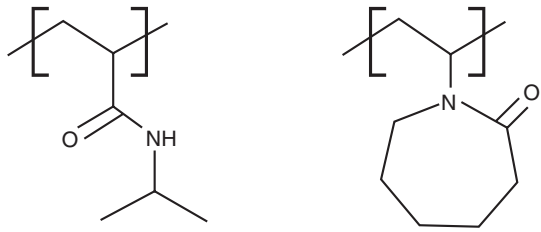
This basic description of UCST behavior in water based on polymer–polymer interactions does not, however, explain the UCST transition of, for example, poly(ethylene oxide) at temperatures beyond the boiling point of water under pressurized conditions. Instead, this UCST transition of poly(ethylene oxide) in superheated water is not related to a major change in polymer properties or polymer association, but rather to a change in solvent properties; that is, under superheated conditions the polarity of water decreases making it a better solvent for poly(ethylene oxide) leading to dissolution of the precipitated polymer chains. A similar change in solvent properties, that is a decrease in polarity upon heating, occurs in a wide variety of alcohol–water mixtures based on the non-ideal mixing behavior of such solvent combinations (Franks and Ives, 1966). Based on this phenomenon, a variety of polymers, including PMMA and poly(2-oxazoline)s, have been reported to undergo UCST phase transitions in alcohol–water mixtures in a temperature range from 0 to 100°C (Lambermont-Thijs *et al.*, 2010; Piccarolo and Titomanilo, 1982).

2.3 Key types of temperature-responsive polymers in aqueous solution

A wide variety of polymers are known that exhibit thermoresponsive behavior in aqueous solution as has been covered in excellent recent reviews on LCST polymers (Liu *et al*., 2009; Roy *et al*., 2013) and UCST polymers (Seuring and Agarwal, 2012a). In general, all water-soluble polymers exhibit LCST behavior in water as the enthalpy of hydration becomes less and the loss of entropy for hydrating water molecules increases with increasing temperature. However, not all polymers exhibit LCST behavior in water at ambient pressure, that is, in between 0°C and 100°C. Therefore, a subtle balance of hydrophilic and hydrophobic groups needs to be present in the polymer structure. Frequently observed hydrophilic moieties are amides and ethers while short aliphatic groups constitute the majority of hydrophobic moieties. In this section a non-comprehensive overview will be given of the most important types of LCST polymers (Sections 2.3.1–2.3.3) and UCST polymers (Section 2.3.4). In addition, the main synthetic procedures for the synthesis of these polymers will briefly be addressed.

2.3.1 Poly(acrylamide)s and poly(vinyl amide)s

The most commonly studied and first reported thermoresponsive polymer in aqueous solution is poly(*N*-isopropylacrylamide) (PNIPAM) (Fig. 2.5) (Aoshima and Kanaoka, 2008; Aseyev *et al*., 2006; Scarpa *et al*., 1967; Schild, 1992). The popularity of PNIPAM is not only based on the LCST that lies in between body and room temperature (LCST ~32°) making it very interesting for biomedical applications, but also on the robust phase behavior. The position of the LCST of PNIPAM with regard to polymer concentration does not shift with variations in chain length. Furthermore, small variations in polymer concentration and solution pH do not induce strong changes in T_{CP}. Shortly after this first report on the LCST transition of PNIPAM, the LCST of poly(*N*-vinyl caprolactam) (PVCL) (Fig. 2.5) was reported to be ~31°C (Aseyev *et al*., 2006; Kirsh, 1993; Solomon *et al*., 1968). Furthermore, both PNIPAM and PVCL have been reported to be similarly biocompatible making them ideal candidates for biomedical applications (Vihola *et al*., 2005). In regard to the very similar properties of both polymers, it is quite surprising that PNIPAM is considered to be the gold standard of thermoresponsive polymers, especially for biomedical applications, and that PVCL has never reached or even come close to such a status. Both PNIPAM and PVCL also share a common drawback for use in biomedical applications and that is their rather high glass transition temperatures (T_g ~ 140–150°C), which has been reported to lead to vitrification of the highly concentrated



PNIPAM PVCL

2.5 Structures of poly(*N*-isopropylacrylamide) (PNIPAM) and poly(*N*-vinyl caprolactam) (PVCL).

polymer phase during phase separation, potentially inducing hysteresis between the heating and cooling transitions (Meeussen *et al.*, 2000; van Durme *et al.*, 2004).

The polymerization of both the *N*-isopropylacrylamide (NIPAM) and *N*-vinylcaprolactam (VCL) monomers can be achieved by free radical polymerization of the vinyl group using a common radical initiator, such as azobisisobutyronitrile (AIBN). In recent years, the controlled radical polymerization (CRP) of both monomers has also been developed resulting in polymers of defined and narrow molecular weight distribution and defined end-groups. Such defined polymers are a prerequisite for biomedical applications while the control over end-groups enables straightforward modification and conjugation towards biological species. In this regard it is important to note that the vinyl group of NIPAM is activated by the amide group while the vinyl group in VCL is much less activated due to reversal of the amide moiety. As a result, the choice and optimization of CRP method will be quite different for both monomers as exemplified on the basis of reversible-addition fragmentation chain-transfer (RAFT) polymerization. The RAFT polymerization of NIPAM can be best performed with RAFT-agents comprising dithiobenzoate or trithiocarbonate groups while the RAFT polymerization of vinyl amides, including VCL, does not go well with these RAFT-agents, but works best with xanthates as RAFT-agent (Lowe and McCormick, 2007). Finally, anionic polymerization methods can be used for the direct polymerization of VCL as well as for the polymerization of protected NIPAM derivatives (Ishizone and Ito, 2002).

The versatility of the radical polymerization mechanism of NIPAM and VCL provides straightforward access to a wide range of copolymers based on the large variety of vinyl monomers that are commercially available. As such, the T_{CP} of PNIPAM and VCL can be easily controlled and tuned by the preparation of statistical copolymers with inert comonomers having higher or lower hydrophilicity to increase or decrease the T_{CP}, respectively

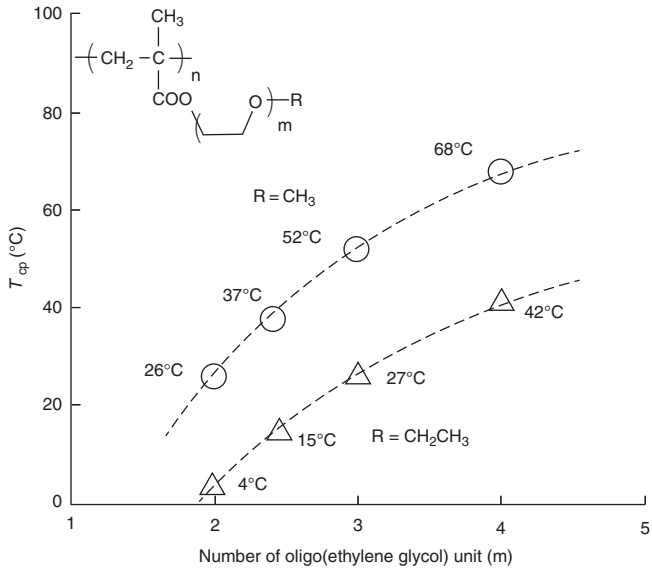
(Dimitrov *et al*., 2007; Schild, 1992). Furthermore, switchable comonomers can be introduced resulting in the formation of multistimuli-responsive copolymers. Examples of such multiresponsive polymers include pH-responsive materials based on the incorporation of pH-switchable side chains, such as tertiary amines or carboxylic acids, and UV responsive materials based on the incorporation of UV-switchable side groups, such as azobenzene or spiropyran moieties (Dimitrov *et al*., 2007; Roy *et al*., 2010, 2013).

Of course, the temperature-responsive behavior of poly(acrylamide)s and poly(vinyl amide)s is not limited to the exact structures of PNIPAM and PVCL, and also analogous polymer structures have been reported to undergo temperature-induced phase separation upon heating in aqueous solution, such as poly(*N*-cyclopropylacrylamide) (Kuramoto and Shishido, 1998), poly(*N,N*-diethylacrylamide) (Lessard *et al*., 2001), poly(*N*-vinyl piperidone) (Ieong *et al*., 2011) and various substituted poly(*N*-vinyl pyrrolidone)s (Yan *et al*., 2010).

2.3.2 Poly(oligo ethylene glycol (meth)acrylate)s

Despite the beneficial properties of PNIPAM, there has been an ongoing search for alternative LCST polymers with further improved properties, especially with regard to (1) lowering the T_g to avoid formation of a glassy polymer phase and suppressing the occurrence of hysteresis and (2) easier synthesis by CRP since, in practice, CRP of NIPAM can be cumbersome. The most studied class of alternatives to PNIPAM in recent years are the poly(oligo ethylene glycol (meth)acrylate)s (POEG(M)A)s consisting of a poly(meth)acrylate backbone furnished with oligo ethylene glycol side chains (Lutz, 2008; Weber *et al*., 2012). The thermoresponsive behavior of such POEGMAs, prepared by living anionic polymerization, was first reported in 2003, demonstrating the tunability of the T_{CP} by variation of the number of ethylene glycol repeat units and also, more recently, by systematic variation of the oligo ethylene glycol chain-end functionality (Fig. 2.6) (Han *et al*., 2003; Ishizone *et al*., 2008).

In 2006, Lutz and coworkers reported the versatility of the CRP of oligo ethylene glycol methacrylates (OEGMAs) using atom transfer radical polymerization (ATRP) not only for their homopolymerization, but especially for their copolymerization (Lutz and Hoth, 2006a). The copolymerization of OEGMA monomers with short and long oligo ethylene glycol side chains, that is, corresponding to POEGMA homopolymers with low and high T_{CP} respectively, allows accurate fine-tuning of the T_{CP} of the copolymers in between the two extremes. A detailed comparison of such POEGMAs and PNIPAM revealed that both polymers have very similar thermoresponsive behavior with regard to salt, molecular weight and concentration dependence

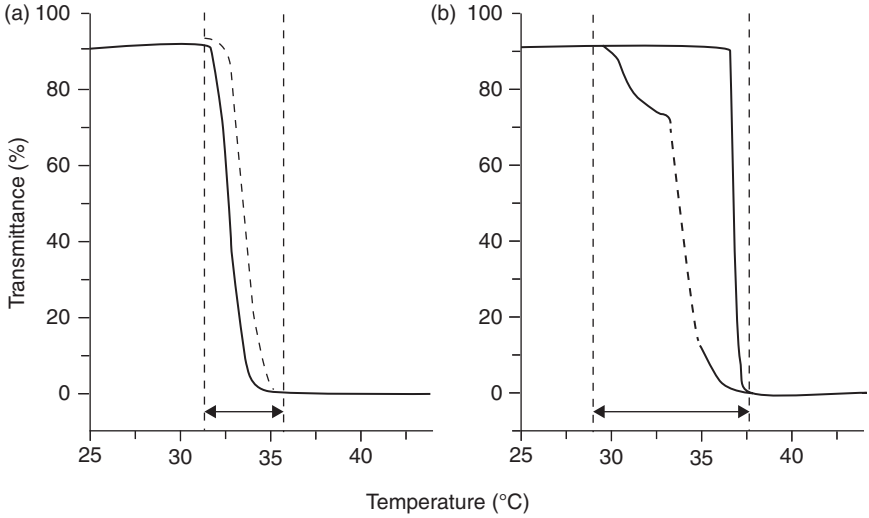


2.6 Variation of cloud point temperature (T_{CP})of poly(oligo ethylene glycol methacrylate)s (POEGMA)s as a function of the number of oligo(ethylene oxide) units (m) and end-group functionality (Ishizone *et al.,* 2008). (*Source*: Reprinted with permission from ACS.)

of the T_{CP} (Lutz *et al*., 2006b). However, the POEGMA does not show significant hysteresis in between the heating and cooling cycles while PNIPAM does show such hysteresis, ascribed to the high T_g (Fig. 2.7), *vide supra*. This ground breaking work in combination with the commercial availability of the OEGMA monomers and straightforward CRP made these POEGMAs very popular thermoresponsive polymers, which nowadays strongly compete with PNIPAM (Lutz, 2008; Lutz, 2011; Weber *et al*., 2012).

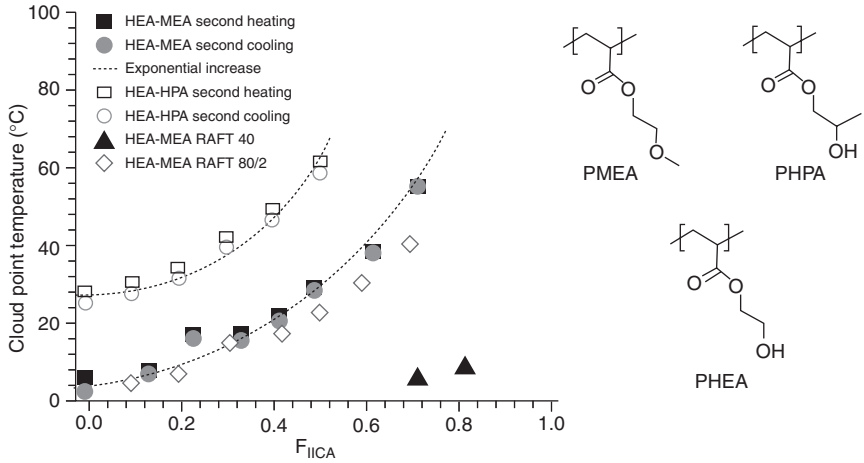
Similarly to the poly(acrylamide)s and poly(vinyl amide)s, POEGMA can be prepared by free radical polymerization, CRP and anionic polymerization, whereby the latter two methods result in well-defined polymer structures with defined end-groups. Even though CRP of OEGMA can be performed by ATRP and RAFT polymerization (Becer *et al*., 2008; Lutz and Hoth, 2006a), the methacrylate obstructs OEGMA homopolymerization by nitroxide mediated polymerization. This can, however, be overcome by copolymerization with a minor amount of styrenic comonomer that enables good control over the polymerization (Charleux *et al*., 2005; Lessard *et al*., 2012).

A related class of thermoresponsive LCST polymers is the poly(oligo ethylene glycol acrylate)s (POEGAs) in which the poly(methacrylate) polymer backbone is replaced by a poly(acrylate) backbone. The latter is more flexible



2.7 Transmittance plotted versus temperature during heating (solid lines) and cooling (dashed lines) of aqueous solutions of POEGMA (a) and PNIPAM (b) (Lutz *et al.,* 2006b). (*Source*: Reprinted with permission from the ACS.)

and less hydrophobic resulting in a T_{CP} that is ~20°C higher when keeping the oligo ethylene glycol side-chain length and chain-end functionality the same, that is, the T_{CP} of poly(diethylene glycol methyl ether methacrylate) (PmDEGMA) is ~25°C while the T_{CP} of poly(diethylene glycol methyl ether acrylate) (PmDEGA) is ~45°C. In recent years POEGAs have gained significant interest due to their polymerizability with anionic polymerization, ATRP, RAFT and nitroxide mediated polymerization (NMP) (Lessard and Maric, 2008; Skrabania *et al.*, 2007). However, the 'early' reports on thermoresponsive POEGAs are based on the copolymerization of a defined oligoethylene glycol acrylate (OEGA) monomer with a larger OEGA comonomer having a side-chain length distribution. Only recently was the full potential of OEGA monomers for the preparation of defined thermoresponsive polymers unveiled by the copolymerization of very similar defined monomers, such as 2-methoxyethyl acrylate (MEA) or 2-hydropropyl acrylate (HPA) with 2-hydroxyethyl acrylate (HEA). Based on the very low T_{CP} of short poly(methoxyethyl acrylate)s (PMEA) (longer PMEA is even water-insoluble) and poly(hydropropyl acrylate) (PHPA) and the high water-solubility of poly(hydroxyethyl acrylate) (PHEA), these monomer combinations provide access to defined copolymers with a T_{CP} tunable in between 0 and 100°C using NMP, ATRP or RAFT polymerization (Fig. 2.8) (Hoogenboom *et al.*, 2009a, 2012; Lavigueur *et al.*, 2011; Steinhauer *et al.*, 2010).



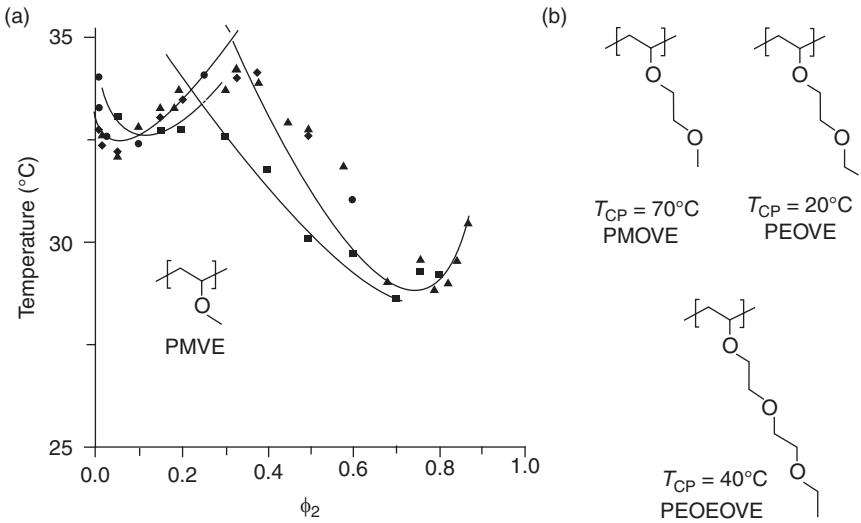
2.8 Cloud point temperature of HEA-MEA and HEA-HPA copolymers prepared by NMP or RAFT polymerization as function of HEA fraction (F_{HEA}) (Hoogenboom *et al.,* 2012). (*Source*: Reprinted with permission from the RSC.)

The thermoresponsive behavior of POEG(M)As can be further tuned by the copolymerization with other (meth)acrylate comonomers to tune the hydrophilicity/hydrophobicity of the polymer leading to higher/lower T_{CP} (Dimitrov *et al.*, 2007; Roy *et al.*, 2010, 2013). Similar to PNIPAM and PVCL, multiresponsive POEG(M)As can be obtained by the incorporation of comonomers that respond to other stimuli, such as pH or UV-irradiation (Dimitrov *et al.*, 2007; Roy *et al.*, 2010, 2013).

2.3.3 Other polymers

Besides the main classes of thermoresponsive polymers covered in the previous two sections, a wide variety of other polymers has been reported to exhibit LCST behavior in water, ranging from relatively simple poly(propylene oxide)-co-poly(ethylene oxide) copolymers (Alred, 1994) to rather complex polypeptides with a repeating Val-Pro-Gly-Val-Gly (VPGVG) motif inspired by elastin (Urry, 1984). Similarly, all other (co)polymers with the correct balance between hydrophilicity and hydrophobicity will undergo a LCST phase transition upon heating. In this section, we will highlight three of such other classes of thermoresponsive polymers, namely poly(vinyl ether)s, poly(2-oxazoline)s and poly(phosphoester)s.

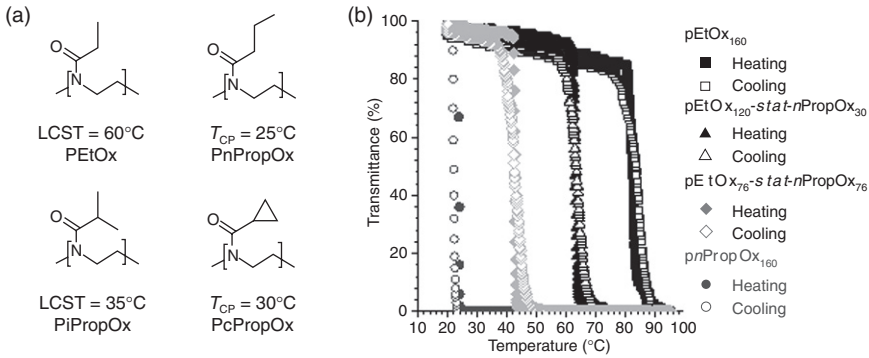
The LCST behavior of poly(methyl vinyl ether) (PMVE) was reported in 1971 with a T_{CP} of 34°C (Aoshima and Kanaoka, 2008; Aseyev *et al.*, 2006; Horne *et al.*, 1971), which was shortly after the first reports on the LCST



2.9 (a) Demixing temperature from differential scanning calorimetric measurements (0.1 K min^{-1}) for aqueous solutions of PMVE (squares: PMVE with M_n = 11 kDa, $Đ$ = 2.5; triangles: PMVE with M_n = 19, $Đ$ = 7.8) as a function of polymer weight fraction (Schäfer-Soenen *et al.*, 1997). (*Source*: Reprinted with permission from ACS.) (b) Structures and cloud point temperatures (T_{CP}) of various poly(vinylether)s (Aoshima, 1992). (*Source*: Reprinted with permission from Elsevier.)

behavior of PNIPAM. The phase behavior of PMVE is rather unusual with two minima in the phase diagram, one dependent on the polymer molar mass similar to PVCL and one at higher polymer concentration of which the position is independent of polymer molar mass as is also the case for PNIPAM (Fig. 2.9; Schäfer-Soenen *et al.*, 1997). In addition to PMVE, the thermoresponsive behavior of various ethylene glycol modified poly(vinyl ether)s (PMOVE, PEOVE and PEOEOVE) has been reported, whereby the T_{CP} can be altered by variation of the ethylene glycol length and end-group as illustrated in Fig. 2.9 (Aoshima *et al.*, 1992).

Although vinyl ethers can be polymerized by free radical polymerization, this commonly results in slow polymerization and low polymerization degrees due to insufficient activation of the vinyl group by the ether moiety. Therefore, the preparation of (defined) poly(vinyl ether)s is commonly performed by (living) cationic vinyl polymerization, which is enabled by stabilization of the cationic propagating species by the ether group. As such, fast uncontrolled polymerization can be obtained with strong cationic initiators, such as stannyl tetrachloride or boron trifluoride (Schröder, 2000). Modification of the cationic polymerization procedure to obtain an equilibrium between cationic propagating species and dormant covalent species



2.10 (a) Structures and cloud point temperatures (T_{CP}) of various poly(2-oxazoline)s. (b) Transmittance versus temperature plots for various poly(2-oxazoline)s consisting of 2-ethyl-2-oxazoline (EtOx) and 2-n-propyl-2-oxazoline (PropOx) (Hoogenboom, 2008). (*Source*: Reprinted with permission from the RSC.)

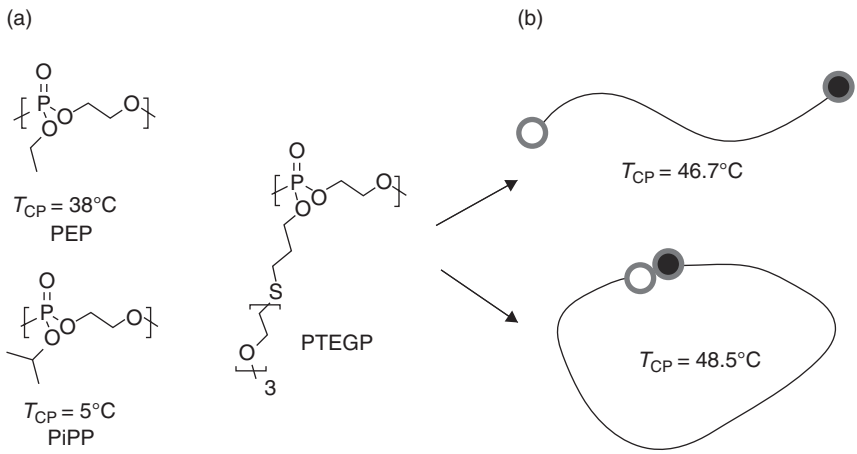
enables living cationic polymerization of vinyl ethers leading to well-defined polymers with defined end-groups (Kojima *et al.*, 1989; Miyamoto *et al.*, 1984).

A recently emerging class of thermoresponsive polymers are the poly(2-oxazoline)s, which are synthetic poly(amide)s comprising a tertiary amide group in the repeat unit and a variable side chain as shown in Fig. 2.10 (Hoogenboom, 2009; Hoogenboom and Schlaad, 2011). Poly(2-oxazoline)s with methyl side chains are very hydrophilic and do not show a LCST phase transition in water, but extending the hydrophobic side-chain length to ethyl or propyl induces thermoresponsive LCST behavior. The LCST of poly(2-ethyl-2-oxazoline) has been reported to be ~60°C (Lin *et al.*, 1988), but has also been demonstrated to be strongly dependent on both molecular weight and concentration (Christova *et al.*, 2003; Hoogenboom *et al.*, 2008). Further extending the side-chain length to n-propyl leads to polymers with a T_{CP} of ~25°C (Hoogenboom *et al.*, 2008; Park and Kataoka, 2007) while variation of the propyl side chain provides further control over the T_{CP} as demonstrated by the T_{CP} of ~35°C and ~30°C for poly(2-oxazoline)s with isopropyl and cyclopropyl side chains, respectively (Bloksma *et al.*, 2011; Uyama and Kobayashi, 1992). Copolymerization of the different 2-oxazoline monomers allows facile tuning of the T_{CP} and copolymers of 2-ethyl-2-oxazoline (EtOx) and 2-n-propyl-2-oxazoline (nPropOx) with a T_{CP} close to body temperature were demonstrated to be promising alternatives to PNIPAM, showing similar concentration dependence on the T_{CP} while no significant hysteresis was present; this was ascribed to the lower T_g and the absence of intramolecular hydrogen bonding in the collapsed state when compared to PNIPAM (Hoogenboom *et al.*, 2008).

Poly(2-oxazoline)s can be prepared by living cationic ring-opening polymerization of the 2-oxazoline monomers utilizing an electrophilic initiator, such as methyl tosylate or methyl triflate. Attack of the monomer onto this initiator leads to the formation of a cationic oxazolinium species and subsequent monomer attack leads to ring-opening while the newly added monomer ends up as a cationic oxazolinium chain end. As such, well-defined polymers can be obtained and the chain-end functionalities can be controlled during initiation and termination (Aoi and Okada, 1996).

A final class of thermoresponsive LCST polymers that is highlighted in this chapter are the poly(phosphoester)s (Iwasaka, 2011). Poly(phosphoester)s comprise hydrolysable phosphoester groups in the main-chain and represent a relatively new class of biocompatible and biodegradable polymers (Zhao, 2003). When the hydrophilicity of the phosphoester moieties is counterbalanced by hydrophobic alkoxy side chains, such as ethyloxy or isopropyloxy, thermoresponsive poly(phosphoester)s are obtained with a T_{CP} of 38°C or 5°C, respectively, as depicted in Fig. 2.11 (Iwasaka, 2011; Iwasaka *et al*., 2007). Furthermore, the T_{CP} linearly depends on the monomer feed ratio in copolymers of these two monomers. In addition, it was recently reported that poly(phosphoester)s with tri(ethylene gycol) thio ether side chains (PTEGP, see Fig. 2.11) also exhibit LCST behavior and that the T_{CP} depends on the polymer chain architecture; that is, the T_{CP} of a cyclic polymer was found to be higher compared to the linear analogue (Fig. 2.11) (Yuan *et al*., 2012).

Poly(phosphoester)s are commonly prepared by ring-opening polymerization of cyclic 2-alkoxy-2-oxo-1,3,2-dioxaphopholanes (cyclic phosphoesters) in the presence of stannous octoate ($Sn(Oct)_2$) as catalyst and an



2.11 (a) Structures and reported cloud point temperatures (T_{CP}) of thermoresponsive poly(phosphoester)s. (b) Effect of polymer architecture of PTEGP on the T_{CP} (Yuan *et al.,* 2012).

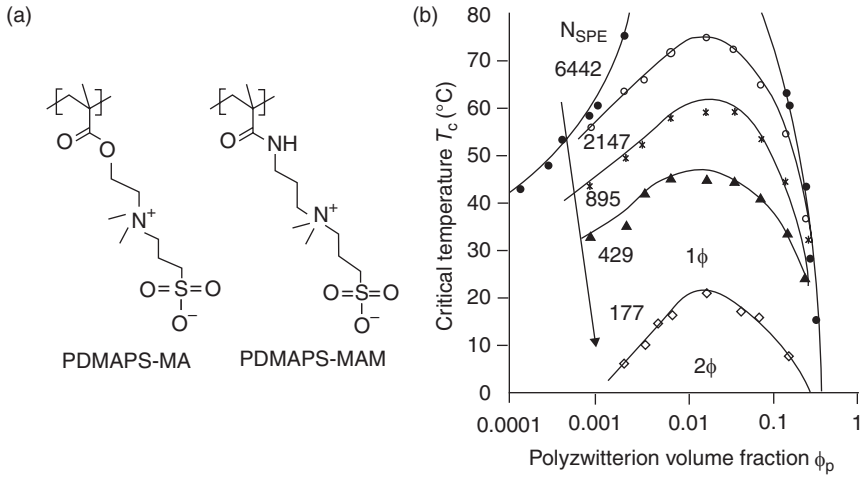
alcohol as initiator. In recent years, significant progress has been made in replacing this $Sn(Oct)_2$ catalyst with organic bases, such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) or 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD), yielding well-defined, metal-free poly(phosphoesther)s (Clement *et al.*, 2012; Iwasaka and Yamaguchi, 2010).

2.3.4 UCST polymers

Thermoresponsive polymers with UCST behavior in water are quite rare, especially in comparison to the rather generally observed LCST behavior. As explained in Section 2.2.2, UCST behavior in water under ambient pressure conditions results from strong supramolecular polymer–polymer interactions, such as electrostatic interactions or hydrogen bonding. Furthermore, UCST behavior can result from a change in solvent quality upon heating, mostly observed in non-ideal alcohol–water solvent mixtures. In this section, the most important classes and types of UCST polymers will be discussed.

The best known type of polymers with UCST behavior in water are the so-called poly(betaine)s, which are zwitterionic polymers comprising both positive and negative charges in every repeat unit (Kudaibergenov *et al.*, 2006). As such, strong polymer–polymer interactions are present based on electrostatic interactions leading to collapsed structures that are more hydrophobic than the solubilized polymer chains at elevated temperatures due to charge compensation and the release of counterions into solution. The most common poly(betaine)s with UCST behavior are poly(2-dimethyl(methacryloxyethyl) ammonium propane sulfonate) (PDMAPS-MA) and poly(3-(N-(3-methacrylamidopropyl)-N,N-dimethyl) ammonium propane sulfonate (PDMAPS-MAM) as depicted in Fig. 2.12 (Huglin and Radwan, 1991; Mary *et al.*, 2007; Schulz *et al.*, 1986). The phase behavior of PDMAPS-MA was reported to be highly dependent on the polymer chain length, whereby the UCST transition increases with increasing chain length as may be expected from the enhanced number of electrostatic interactions that increase the polymer–polymer interactions (Fig. 2.12) (Mary *et al.*, 2007).

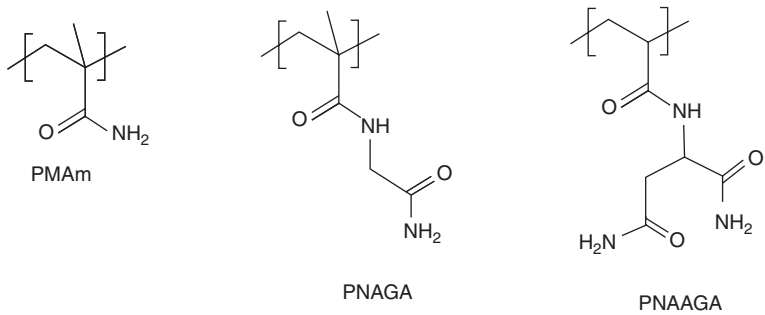
Alternatively, UCST behavior in water can be achieved based on the interactions between a polyelectrolyte with multivalent counterions as has been described for solutions containing poly(dimethylamino ethyl methacrylate) with the trivalent anion $[Co(CN)_6]^{3-}$ (Plamper *et al.*, 2007). Despite the hydrogelation of polyelectrolytes in the presence of multivalent ions being commonly known, such as the gelation of alginate in the presence of calcium(II), there are few studies focusing on the UCST type phase transitions of such hydrogels or polymer solutions. The synthesis of such vinyl polymers can be performed by radical polymerization, as discussed in Section 2.3.1.



2.12 (a) Structure and cloud point temperatures (T_{CP}) of common poly(betaine)s. (b) Phase diagram for the UCST behavior of poly(2-dimethyl(methacryloxyethyl) ammonium propane sulfonate) (PDMAPS-MA) in water as a function of the degree of polymerization (N_{SPE}) (Mary *et al.*, 2007). (*Source*: Reprinted with permission from the ACS.)

Polymers with UCST behavior in water, based on attractive hydrogen bonding interactions, have recently gained significant interest, mostly based on the discovery that primary amide groups present in the polymer side chains can induce UCST behavior on the condition that no ionic impurities are present in the polymer due to partial hydrolysis of the side chains (Seuring and Agarwal, 2012a). Figure 2.13 depicts some recently reported homopolymers that exhibit such hydrogen bonding-based UCST behavior including poly(methacrylamide) (PMAm) (Seuring and Agarwal, 2012b), poly(N-acryloylglycinamide) (PNAGA) (Glatzel *et al.*, 2010; Seuring and Agarwal, 2010) and poly(N-acryloylasparaginamide) (PNAAGA) (Glatzel *et al.*, 2011). Furthermore, copolymers of poly(acrylamide) with hydrophobic comonomers also reveal UCST behavior in water (Seuring and Agarwal, 2012b) and replacing the primary amide group with a primary ureido-functionality also yields UCST thermoresponsive polymers in water (Shimada *et al.*, 2011).

The final important class of thermoresponsive polymers with UCST behavior in alcohol–water mixtures consists of hydrophobic polymers that comprise good hydrogen bond accepting moieties, such as ester groups, ether groups or tertiary amides to interact with the solvent. Reported examples include poly(methyl (meth)acrylate)s (Can *et al.*, 2010; Hoogenboom *et al.*, 2009b; Piccarolo and Titomanilo, 1982), poly(2-oxazoline)s (Lambermont-Thijs *et al.*, 2010) and POEGMAs (Roth *et al.*, 2011).



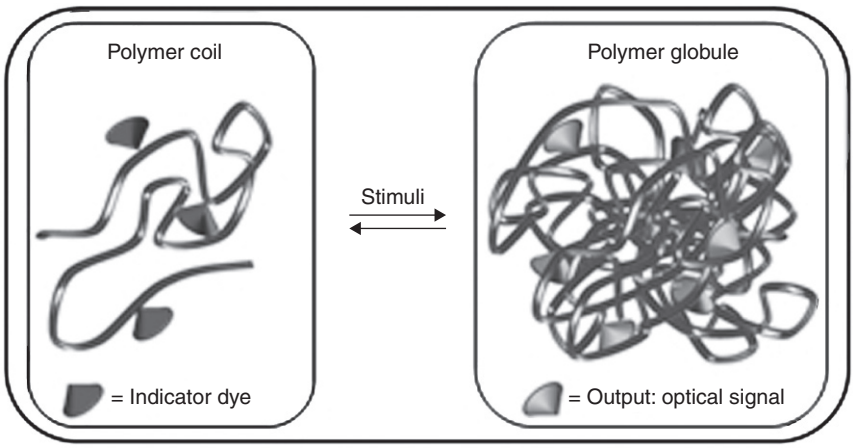
2.13 Structure of polymers that exhibit hydrogen bonding-based UCST behavior in water.

2.4 Selected applications of thermoresponsive polymers

Thermoresponsive polymers provide a promising basis for the development of smart materials. In this section, selected recent examples will be discussed to highlight this potential, focusing on the more common LCST polymers. For more comprehensive overviews of the use of thermoresponsive polymers for biomedical applications, the reader is referred to a number of recent review articles (De las Heras Alarcon *et al.*, 2005; Schmaljohann, 2006; Ward and Theoni, 2011).

The LCST transition of a polymer is accompanied by (partial) dehydration of the polymer chains, which has been applied for the development of polymeric temperature sensors. The incorporation of a solvatochromic dye molecule in the polymer side chain provides a direct read-out of the temperature transition of the polymer by a change in color or fluorescence resulting from the change in the polarity of the microenvironment of the dye, as illustrated in Fig. 2.14 (Pietsch *et al.*, 2011). Such polymeric thermometers were recently developed for accurate local temperature determination inside living cells, for which the read-out was changed to fluorescence lifetime rather than emission wavelength or intensity in order to have a more robust sensor (Okabe *et al.*, 2012). Furthermore, dual sensors have been developed by combining a LCST polymer with a pH-responsive solvatochromic dye, which led to a sensor that allows determination of both the solution temperature and pH by a single UV-measurement (Pietsch *et al.*, 2009).

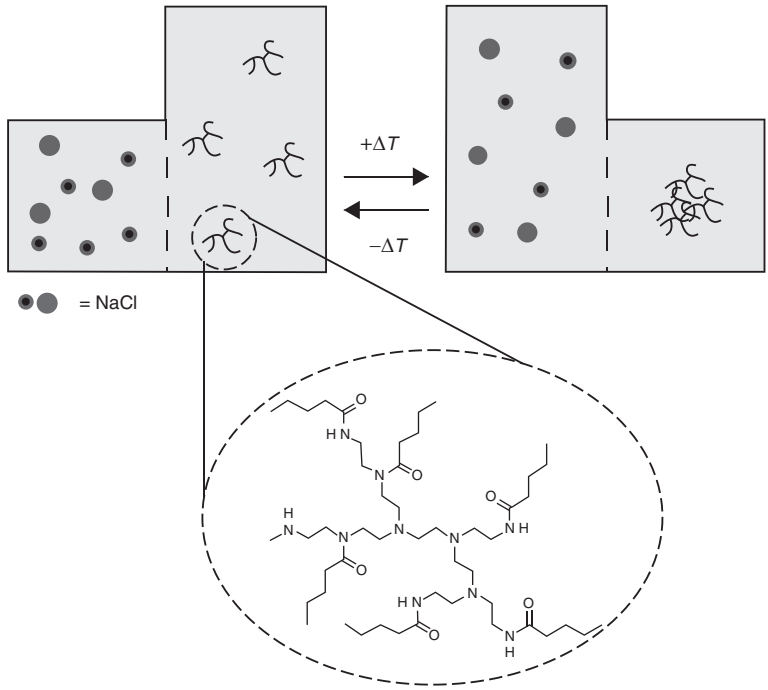
The change in polymer conformation during the LCST transition can also be applied to control the proximity of side-chain functionalities. In a recent report, this characteristic of the polymer phase transition was exploited to control the binding kinetics of mannose-functionalized PNIPAM hydrogel



2.14 Schematic representation of the concept of polymeric sensors based on a polymer phase transition as thermoresponsive structure and solvatochromic dyes to provide a visual or fluorescence read-out signal of the change in the polarity of the microenvironment (Pietsch *et al.,* 2011). (*Source*: Reprinted with permission from the RSC.)

nanoparticles to sugar binding proteins, so-called lectins (Hoshino *et al*., 2012). As may be expected, the binding of the PNIPAM nanoparticles was stronger in the hydrated state below the T_{CP} compared to the collapsed state, which can be ascribed to the higher mobility and availability of the mannose units. However, the binding was found to be even stronger in the phase transition regime where partial dehydration leads to contraction of the nanoparticles and, thus, close proximity of the mannose groups while apparently they are still mobile enough to bind to the lectin. As such, variation of temperature might lead to on and off switching of the nanoparticle–lectin binding.

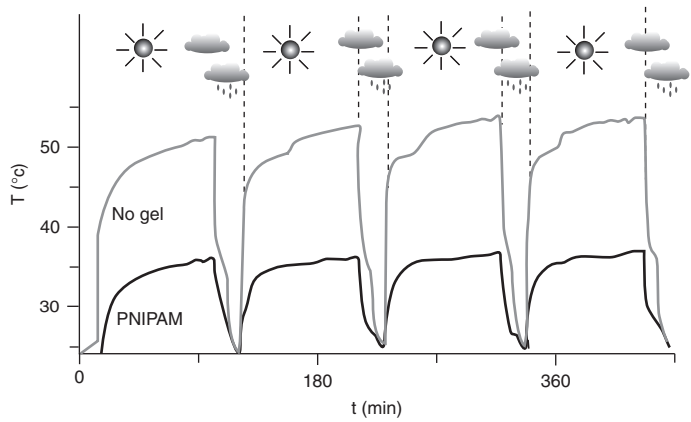
In addition to applications based on the change in the polymer chain conformation, the change in effective polymer concentration upon LCST dehydration and aggregation of the polymer chains has been applied to control osmotic strength of a polymer solution and to induce a reversible movement of water through a membrane (Fig. 2.15) (Noh *et al*., 2012). Acylated branched poly(ethylene imine)s were utilized as thermoresponsive polymers exhibiting tunable T_{CP} in between 20°C and 54°C (Fig. 2.15). The osmotic flux experiment was optimized and developed with the n-butyl acetylated polymer having a T_{CP} of ~30°C. A solution of this polymer was placed in contact with a lower concentration NaCl solution via a semipermeable cellulose trifluoroacetate membrane that allows only water to pass, leading to osmotic water flux from the NaCl solution to the polymer solution at 21°C



2.15 Schematic representation to the reversible temperature-induced control over osmotic flux (top) and the structure of the utilized thermoresponsive butylated branched poly(ethylene imine) (Noh *et al.*, 2012).

(Fig. 2.15). Upon heating the system to 55°C, the polymer undergoes the LCST transition leading to aggregation and, thus, a lower effective polymer concentration resulting in reversed osmotic flux. This process was demonstrated to be fully reversible in three repetitive cycles and it has been suggested that such a system might find potential use for the desalination of seawater.

In a related study, the application of thermoresponsive polymers for the collection of water from fogs was reported (Yang *et al.*, 2013). Cotton fibers were modified with a thermoresponsive PNIPAM coating resulting in a thermoresponsive cotton with a T_{CP} ~32°C. The water uptake of these PNIPAM-modified cotton fibers was highly temperature responsive revealing high water uptake at 24°C (~350 wt%) and almost no water uptake at 33°C (~50 wt%) and higher temperatures. Furthermore, it was demonstrated that the water uptake can be reversibly switched, thereby enabling water absorption from the atmosphere at lower temperatures, such as during night, and release (i.e. collection) upon heating, such as during daytime.



2.16 Temperature variation inside a miniaturized building during alternating rain and sun exposure cycles with and without a PNIPAM hydrogel on the roof (Rotzetter *et al.,* 2012). (*Source*: Reprinted with permission from Wiley.)

Similar control over water absorption and release by a cross-linked PNIPAM hydrogel has been proposed for passive cooling of buildings (Rotzetter *et al*., 2012). During rain the hydrogel is below the T_{CP} of the PNIPAM hydrogel leading to water absorption, while in the sun, the temperature rises beyond the T_{CP} and the water is expelled from the hydrogel. Evaporation of the released water leads to passive cooling as has been clearly demonstrated by measuring the temperature of a miniaturized building with and without such a PNIPAM hydrogel on the roof during artificial sun and rain cycles (Fig. 2.16).

Thermoresponsive polymers also provide opportunities for the preparation of thermoresponsive hydrogels (Klouda and Mikos, 2008; van Vlierberghe *et al*., 2011; Ward and Theoni, 2011). Highly concentrated thermoresponsive polymer solutions (commonly > 10 wt%) undergo LCST-based temperature-induced gelation upon heating due to the partial dehydration of the polymer chains leading to the formation of a physically cross-linked polymer network that solidifies the solution. Very recently, however, a thermoresponsive poly(isocyanide) bearing ethylene glycol-functionalized peptidic side chains was reported to undergo LCST driven, temperature-induced hydrogelation at a concentration as low as 0.006 wt% (Kouwer *et al*., 2013). This highly efficient hydrogelation was ascribed to the high stiffness of the helical polymer chains in combination with the formation of physical cross-links by bundling of individual helical polymer chains in larger fibers upon partial dehydration of the side chains. Surprisingly, these hydrogels revealed shear-thickening behavior and their properties closely resembled the properties of naturally occurring gels based in intermediate actin filaments.

2.5 Conclusion

Thermoresponsive polymers have gained significant interest in the past decade, especially those that undergo a temperature-induced phase transition in aqueous solutions. Water-soluble polymers generally undergo an LCST phase transition in water and, thus, the majority of reports focus on such LCST polymers. In recent years, the gold standard of LCST polymers, namely PNIPAM, has lost terrain to other alternative thermoresponsive polymers of which the T_{CP} can be more easily tuned and that show less hysteresis between heating and cooling cycles, including POEG(M)As and poly(2-oxazoline)s. In contrast to LCST polymers, there is only a relatively small number of polymers reported that undergo a UCST transition in aqueous solution. Recent progress includes the development of UCST polymers with primary amide side chains that strongly interact by hydrogen bonding, but the UCST transition is strongly affected by minor ionic impurities as well as the ionic strength of the solution. Therefore, the major challenge in this field is the development of polymers with a robust UCST transition in aqueous solution.

The large application potential of LCST polymers is currently being explored for a wide variety of smart materials, ranging from biomedical applications, to sensors, water collection and energy efficient buildings. However, further in-depth studies are required before such materials will be able to enter the market.

2.6 Future trends

Future trends are expected to focus on the development of multiresponsive polymers that combine a LCST phase transition with another response, such as redox, pH or the presence of certain analytes. As such, the phase transition can be induced isothermally by the second response parameter to further broaden the application potential for sensors and biomedical applications. Furthermore, the development of novel polymers with UCST behavior will be an important research topic for the coming years as well as the development of applications of UCST polymers. Finally, I am convinced that the application potential of both LCST and UCST polymers for smart materials will be significantly broadened in the near future.

2.7 References

Alred, P. A., Kozlowski, A. Harris, J. M. and Tjerneld, F. (1994) Application of temperature-induced phase partitioning at ambient temperature for enzyme purification, *J Chromatography A*, **659**, 289–298, doi: 10.1385/1–59259–028–4:281.

Aoi, K. and Okada, M. (1996) Polymerization of oxazolines, *Prog Polym Sci*, **21**, 151–208.

Aoshima, S. and Kanaoka, S. (2008) Synthesis of stimuli-responsive polymers by living polymerization: Poly(*N*-isopropylacrylamide) and poly(vinyl ether)s, *Adv Polym Sci*, **210**, 169–208, doi: 10.1007/12_2007_120.

Aoshima, S., Oda, H. and Kobayashi, E. (1992) Synthesis of thermally-induced phase separating polymer with well-defined polymer structure by living cationic polymerization. I. Synthesis of poly(vinyl ether)s with oxyethylene units in the pendant and its phase separation behavior in aqueous solution, *J Polym Sci Part A Polym Chem*, **30**, 2407–2413, doi: 10.1002/pola.1992.080301115.

Aseyev, V. O., Tenhu, H. and Winnik, F. M. (2006) Temperature dependence of the colloidal stability of neutral amphiphilic polymers in water, *Adv Polym Sci*, **196**, 1–85, doi: 10.1007/12_052.

Becer, C. R., Hahn, S., Fijten, M. W. M., Thijs, H. M. L., Hoogenboom, R. and Schubert, U. S. (2008) Libraries of methacrylic acid and oligo(ethylene glycol) methacrylate copolymers with LCST behavior, *J Polym Sci Part A Polym Chem*, **46**, 7138–7147, doi: 10.1002/pola.23018.

Bloksma, M. M., Weber, C., Perevyazko, I. Y., Kuse, A., Baumg€artel,A., Vollrath, A., Hoogenboom, R. and Schubert, U. S. (2011) Poly(2-cyclopropyl-2-oxazoline): From rate acceleration by cyclopropyl to thermoresponsive properties, *Macromolecules*, **44**, 4057–4074, doi: 10.1021/ma200514n.

Can, A., Altuntas, E., Hoogenboom, R. and Schubert, U. S. (2010) Synthesis and MALDI-TOF-MS of PS-PMA and PMA-PS block copolymers, *Eur Polym J*, **46**, 1932–1939, doi: 10.1016/j.eurpolymj.2010.07.003.

Charleux, B., Nicolas, J. and Guerret, O. (2005) Theoretical expression of the average activation–deactivation equilibrium constant in controlled/living free-radical copolymerization operating via reversible termination. Application to a strongly improved control in nitroxide-mediated polymerization of methyl methacrylate, *Macromolecules*, **38**, 5485–5492, doi: 10.1021/ma050087e.

Christova, D., Velichkova, R., Loos, W., Goethals, E. J. and Du Prez, F. (2003) New thermo-responsive polymer materials based on poly(2-ethyl-2-oxazoline) segments, *Polymer*, **44**, 2255–2261, doi: 10.1016/S0032–3861(03)00139–3.

Clement, B., Grignard, B., Koole, L., Jerome, C. and Lecomte, P. (2012) Metal-free strategies for the synthesis of functional and well-defined polyphosphoesters, *Macromolecules*, **45**, 4476–4486, doi: 10.1021/ma3004339.

Cowie, J. M. G. and McEwen, I. J. (1976) Influence of microstructure on the upper and lower critical solution temperatures of poly(methylmethacrylate) solutions, *J Chem Soc Faraday Trans 1: Phys Chem Condensed Phases*, **72**, 526–533, doi: 10.1039/F19767200526.

de las Heras Alarcón, C., Pennadam, S. and Alexander, C. (2005) Stimuli responsive copolymers for biomedical applications, *Chem Soc Rev*, **34**, 276–285, doi: 10.1039/b406727d.

Dimitrov, I., Trzebicka, B., Müller, A. H. E., Dworak, A. and Tsvetanov, C. B. (2007) Thermosensitive water-soluble copolymers with doubly responsive reversibly interacting entities, *Prog Polym Sci*, **32**, 1275–1343, doi: 10.1016/j.progpolymsci.2007.07.001.

Donald, A. M., Windle, A. H. and Hanna, S. (2005) *Liquid Crystalline Polymers*, Cambridge, Cambridge University Press.

Franks, F. and Ives, D.J.G. (1966) The structural properties of alcohol-water mixtures, *Q Rev Chem Soc*, **20**, 1–44, doi: 10.1039/QR9662000001.

Glatzel, S., Badi, N., Päch, M., Laschewsky, A. and Lutz, J.-F. (2010) Well-defined synthetic polymers with a protein-like gelation behavior in water, *Chem Commun*, 4517–4519, doi: 10.1039/c0cc00038h.

Glatzel, S., Laschewsky, A. and Lutz, J.-F. (2011) Well-defined uncharged polymers with a sharp UCST in water and in physiological milieu, *Macromolecules*, **44**, 413–415, doi: 10.1021/ma102677k.

Han, S., Hagiwara, M. and Ishizone, T. (2003) Synthesis of thermally sensitive water-soluble polymethacrylates by living anionic polymerizations of oligo(ethylene glycol) methyl ether methacrylates, *Macromolecules*, **36**, 8312–8319, doi: 10.1021/ma0347971.

Hoogenboom, R. (2009) Poly(2-oxazoline)s: A polymer class with numerous potential applications, *Angew Chem Int Ed*, **48**, 7978–7994, doi: 10.1002/anie.200901607.

Hoogenboom, R. and Schlaad, H. (2011) Bioinspired poly(2-oxazoline)s, *Polymers*, **3**, 467–488, doi: 10.3390/polym3010467.

Hoogenboom, R., Popescu, D., Steinhauer, W., Keul, H. and Moeller, M. (2009a) Nitroxide-mediated copolymerization of 2-hydroxyethyl acrylate and 2-hydroxypropyl acrylate: Copolymerization kinetics and thermoresponsive properties, *Macromol Rapid Commun*, **30**, 2042–2048, doi: 10.1002/marc.200900507.

Hoogenboom, R., Rogers, S., Can, A., Becer, C. R., Guerrero-Sanchez, C., Wouters, D., Hoeppener, S. and Schubert, U. S. (2009b) Self-assembly of double hydrophobic block copolymers in water–ethanol mixtures: from micelles to thermoresponsive micellar gels, *Chem Commun*, 5582–5582, doi: 10.1039/b911858f.

Hoogenboom, R., Thijs, M. H. L., Jochems, M. J. H. C., van Lankvelt, B. M., Fijten, M. W. M. and Schubert, U. S. (2008) Tuning the LCST of poly(2-oxazoline)s by varying composition and molecular weight: alternatives to poly(*N*-isopropylacrylamide)? *Chem Commun*, 5758–5760, doi: 10.1039/b813140f.

Hoogenboom, R., Zorn, A.-M., Keul, H., Barner-Kowollik, C. and Moeller, M. (2012) Copolymers of 2-hydroxyethylacrylate and 2-methoxyethyl acrylate by nitroxide mediated polymerization: kinetics, SEC-ESI-MS analysis and thermoresponsive properties, *Polym Chem*, **3**, 335–342, doi: 10.1039/c1py00344e.

Horne, R. A., Almeida, J. P., Day, A. F. and Yu, N. T. (1971) Macromolecule hydration and the effect of solutes on the cloud point of aqueous solutions of polyvinyl methyl ether: A possible model for protein denaturation and temperature control in homeothermic animals, *J Colloid Interface Sci*, **35**, 77–84, doi: 10.1016/0021–9797(71)90187–1.

Hoshino, Y., Nakamoto, M. and Miura, Y. (2012) Control of protein-binding kinetics on synthetic polymer nanoparticles by tuning flexibility and inducing conformation changes of polymer chains, *J Am Chem Soc*, **134**, 15209–15212, doi: 10.1021/ja306053s.

Huglin, M. B. and Radwan, M. A. (1991) Unperturbed dimensions of a zwitterionic polymethacrylate, *Polym Int*, **26**, 97–104, doi: 10.1002/pi.4990260208.

Ieong, N. S., Redhead, M., Bosquillon, C., Alexander, C., Kelland, M. and O'Reilly, R. K. (2011) The missing lactam-thermoresponsive and biocompatible poly (N-vinylpiperidone) polymers by xanthate-mediated RAFT polymerization, *Macromolecules*, **44**, 886–893, doi: 10.1021/ma1026466.

Ishizone, T. and Ito, M (2002) Synthesis of well-defined poly(*N*-isopropylacrylamide) by the anionic polymerization of *N*-methoxymethyl-*N*-isopropylacrylamide, *J Polym Sci Part A Polym Chem*, **40**, 4328–4332, doi: 10.1002/pola.10518.

Ishizone, T., Seki, A., Hagiwara, M. and Han, S. (2008) Anionic polymerizations of oligo(ethylene glycol) alkyl ether methacrylates: Effect of side chain length and ω-alkyl group of side chain on cloud point in water, *Macromolecules*, **41**, 2963–2967, doi: 10.1021/ma702828n.

Iwasaki, Y. (2011). Modern synthesis and thermoresponsivity of polyphosphoesters. In: *Biomedical Engineering – Frontiers and Challenges*, Reza Fazel (Ed.), Shanghai, InTech, ISBN: 978–953–307–309–5.

Iwasaki, Y., Wachiralarpphaithoon, C. and Akiyoshi, K. (2007) Novel thermoresponsive polymers having biodegradable phosphoester backbones, *Macromolecules*, **40**, 8136–8138, doi: 10.1021/ma0715573.

Iwasaki, Y. and Yamaguchi, E. (2010) Synthesis of well-defined thermoresponsive polyphosphoester macroinitiators using organocatalysts, *Macromolecules*, **43**, 2664–2666, doi: 10.1021/ma100242s.

Kirsh, Y. E. (1993) Water-soluble poly (*N*-vinylamidess): Microstructure, solvation, conformational state and complex formation in aqueous solutions, *Prog Polym Sci*, **18**, 519–542, doi: 10.1016/0079–6700(93)90016–6.

Klouda, L. and Mikos, A. G. (2008) Thermoresponsive hydrogels in biomedical applications – a review, *Eur J Pharm Biopharm*, **68**, 34–45, doi: 10.1016/j.ejpb.2007.02.025.

Kojima, K., Sawamoto, M. and Higashimura, T. (1989) Living cationic polymerization of isobutyl vinyl ether by hydrogen iodide/Lewis acid initiating systems: effects of Lewis acid activators and polymerization kinetics, *Macromolecules*, **22**, 1552–1557, doi: 10.1021/ma00194a008.

Kouwer, P. H. J., Koepf, M., Le Sage, V. A. A., Jaspers, M., van Buul, A. M., Eksteen-Akeroyd, Z. H., Woltinge, T., Schwartz, E., Kitto, H. J., Hoogenboom, R., Picken, S. J., Nolte, R. J. M.,Kudaibergenov, S., Jaeger, W. and Lascheswky, A. (2006) Polymeric betaines: Synthesis, characterization, and application, *Adv Polym Sci*, **201**, 157–224, doi: 10.1007/12_078.

Kuramoto, N. and Shishido, Y. (1998) Property of thermo-sensitive and redox-active poly(*N*-cyclopropylacrylamide-covinylferrocene) and poly(*N*-isopropylacrylamide-co-vinylferrocene), *Polymer*, **39**, 669–675, doi: 10.1016/S0032–3861(97)00334–0.

Lambermont-Thijs, H. M. L., van Kuringen, H. P. C., van der Put, J. P. W., Schubert, U. S. and Hoogenboom, R. (2010) Temperature induced solubility transitions of various poly(2-oxazoline)s in ethanol-water solvent mixtures, *Polymers*, **2**, 188–199, doi: 10.3390/polym2030188.

Lavigueur, C., Garcia, J. G., Hendriks, L., Hoogenboom, R., Cornelissen, J. J. L. M. and Nolte, R. J. M. (2011) Thermoresponsive giant biohybrid amphiphiles, *Polym Chem*, **2**, 333–340, doi: 10.1039/c0py00229a.

Lendlein, A. and Kelch, S. (2002) Shape-Memory Polymers, *Angew Chem Int Ed*, **41**, 2034–2057, doi: 10.1002/1521–3773(20020617).

Lessard, B. and Maric, M. (2008) Nitroxide-mediated synthesis of poly(poly(ethylene glycol) acrylate) (PPEGA) comb-like homopolymers and block copolymers, *Macromolecules*, **41**, 7870–7880, doi: 10.1021/ma800603a.

Lessard, B. H., Ling, E. J. Y. and Maric, M. (2012) Fluorescent, thermoresponsive oligo(ethylene glycol) methacrylate/9-(4-vinylbenzyl)-9H-carbazole copolymers designed with multiple LCSTs via nitroxide mediated controlled radical polymerization, *Macromolecules*, **45**, 1879–1891, doi: 10.1021/ma202648k.

Lessard, D. G., Ousalem, M. and Zhu, X. X. (2001) Effect of the molecular weight on the lower critical solution temperature of poly(N,N-diethylacrylamide) in aqueous solutions, *Can J Chem*, **79**, 1870–1874, doi: 10.1139/v01–180.

Li, M.-H. and Keller, P. (2006) Artificial muscles based on liquid crystal elastomers, *Phil Trans R Soc A*, **364**, 2763–2777, doi: 10.1098/rsta.2006.1853.

Lin, P., Clash, C., Pearce, E. M. and Kwei, T. K. (1988) Solubility and miscibility of poly(ethyl oxazoline), *J Polym Sci Part B Polym Phys*, **26**, 603–619, doi: 10.1002/polb.1988.090260312.

Liu, R., Fraylich, M. and Saunders, B. R. (2009) Thermoresponsive copolymers: from fundamental studies to applications, *Colloid Polym Sci*, **287**, 627–643, doi: 10.1007/s00396–009–2028-x.

Liu, C., Qin, H. and Mather, P. T. (2007) Review of progress in shape-memory polymers, *J Mater Chem*, **17**, 1543–1558, doi: 10.1039/b615954k.

Longenecker, R., Mu, T., Hanna, M., Burke, N. A. D. and Stöer, H. D. H. (2011) Thermally responsive 2-hydroxyethyl methacrylate polymers: Soluble-insoluble and soluble-insoluble-soluble transitions, *Macromolecules*, **44**, 8962–8971, doi: /10.1021/ma201528r.

Lowe, A. B. and McCormick, C. L. (2007) Reversible addition–fragmentation chain transfer (RAFT) radical polymerization and the synthesis of water-soluble (co) polymers under homogeneous conditions in organic and aqueous media, *Prog Polym Sci*, **32**, 283–351, doi: 10.1016/j.progpolymsci.2006.11.003.

Lutz, J.-F. (2008) Polymerization of oligo(ethylene glycol) (meth)acrylates: Toward new generations of smart biocompatible materials, *J Polym Sci Part A Polym Chem*, **46**, 3459–3470, doi: 10.1002/pola.22706.

Lutz, J. F. (2011) Thermo-switchable materials prepared using the OEGMA-platform, *Adv Mater*, **23**, 2237–2243, doi: 10.1002/adma.201100597.

Lutz, J.-F. and Hoth, A. (2006a) Preparation of ideal PEG analogues with a tunable thermosensitivity by controlled radical copolymerization of 2-(2-methoxyethoxy)ethyl methacrylate and oligo(ethylene glycol) methacrylate, *Macromolecules*, **39**, 893–896, doi: 10.1021/ma0517042.

Lutz, J.-F., Akdemir, O. and Hoth, A. (2006b) Point by point comparison of two thermosensitive polymers exhibiting a similar LCST: Is the age of poly(NIPAM) over? *J Am Chem Soc*, **128**, 13046–13047, doi: 10.1021/ja065324n.

Mary, P., Bendejacq, D. D., Labeau, M.-P. and Dupuis, P. (2007) Reconciling low- and high-salt solution behavior of sulfobetaine polyzwitterions, *J Phys Chem B*, **111**, 7767–7777, doi: 10.1021/jp071995b.

Meeussen, F., Nies, E., Berghmans, H., Verbrugghe, S., Goethals, E. and Du Prez, F. (2000) Phase behaviour of poly(*N*-vinyl caprolactam) in water, *Polymer*, **41**, 8597–8602, doi: 10.1016/S0032–3861(00)00255-X.

Mendes, E. and Rowan, A. E. (2013) Responsive biomimetic networks from polyisocyanopeptide hydrogels, *Nature*, **493**, 651–655, doi: 10.1038/nature11839.

Miyamoto, M., Sawamoto, M. and Higashimura, T. (1984) Living polymerization of isobutyl vinyl ether with hydrogen iodide/iodine initiating system, *Macromolecules*, **17**, 265–268, doi: 10.1021/ma00133a001.

Noh, M., Mok, Z., Lee, S. and Kim, H. (2012) Novel lower critical solution temperature phase transition materials effectively control osmosis by mild temperature changes, *Chem Commun*, **48**, 3845–3847, doi: 10.1039/c2cc30890h.

Nord, F. F., Bier, M. and Timasheff, S. N. (1951) Investigations on proteins and polymers. 1V. Critical phenomena in polyvinyl alcohol-acetate copolymer solutions, *J Am Chem Soc*, **73**, 289–293, doi: 10.1021/ja01145a095.

Okabe, K., Inada, N., Gota, C., Harada, Y., Funatsu, T. and Uchiyama, S. (2012) Intracellular temperature mapping with a fluorescent polymeric thermometer and fluorescence lifetime imaging microscopy, *Nat Commun*, **3**, art nr 705, doi: 10.1038/ncomms1714.

Park, J.-S. and Kataoka, K. (2007) Comprehensive and accurate control of thermosensitivity of poly(2-alkyl-2-oxazoline)s via well-defined gradient or random copolymerization, *Macromolecules*, **40**, 3599–3609, doi: 10.1021/ma0701181.

Piccarolo, S. and Titomanlio, G. (1982) Synergism in the swelling and solubility of poly(methyl methacrylate) in presence of ethanol/water mixtures, *Makromol Chem Rapid Commun*, **3**, 383–387, doi: 10.1002/marc.1982.030030602.

Pietsch, C., Hoogenboom, R. and Schubert, U. S. (2009) Soluble polymeric dual sensor for temperature and pH value, *Angew Chem Int Ed*, **48**, 5653–5656, doi: 10.1002/anie.200901071.

Pietsch, C., Hoogenboom, R. and Schubert, U. S. (2010b) PMMA based soluble polymeric temperature sensors based on UCST transition and solvatochromic dyes, *Polym Chem*, **1**, 1005–1008, doi: 10.1039/c0py00162g.

Pietsch, C., Schubert, U. S. and Hoogenboom, R. (2011) Aqueous polymeric sensors based on temperature-induced polymer phase transitions and solvatochromic dyes, *Chem Commun*, **47**, 8750–8765, doi: 10.1039/C1CC11940K.

Pietsch, C., Vollrath, A., Hoogenboom, R. and Schubert, U. S. (2010a) A fluorescent thermometer based on a pyrene-labeled thermoresponsive polymer, *Sensors*, **10**, 7979–7990, doi: 10.3390/s100907979.

Plamper, F. A., Schmalz, A., Ballauf, M. and Müller, A. H. E. (2007) Tuning the thermoresponsiveness of weak polyelectrolytes by pH and light: Lower and upper critical-solution temperature of poly(N,N-dimethylaminoethyl methacrylate), *J Am Chem Soc*, **127**, 14538–14539, doi: 10.1021/ja074720i.

Roth, P. J., Jochum, F. D. and Theato, P. (2011) UCST-type behavior of poly[oligo(ethylene glycol) methyl ether methacrylate] (POEGMA) in aliphatic alcohols: solvent, co-solvent, molecular weight, and end group dependences, *Soft Matter*, **7**, 2484–2492, doi: 10.1039/c0sm01324b.

Rotzetter, A. C. C., Schumacher, C. M., Bubenhofer, S. B., Grass, R. N., Gerber, L. C., Zeltner, M. and Stark, W. J. (2012) Thermoresponsive polymer induced sweating surfaces as an efficient way to passively cool buildings, *Adv Mater*, **24**, 5352–5356, doi: 10.1002/adma.201202574.

Roy, D., Brooks, W. L. A. and Sumerlin, B. S. (2013) New directions in thermoresponsive polymers, *Chem Soc Rev*, **42**, 7214–7243.

Roy, D., Cambre, J. N. and Sumerlin, B. S. (2010) Future perspectives and recent advances in stimuli-responsive materials, *Prog Polym Sci*, **35**, 278–301, doi: 10.1016/j.progpolymsci.2009.10.008.

Saeki, S., Kuwahara, N., Nakata, M. and Kaneko, M. (1976) Upper and lower critical solution temperatures in poly (ethylene glycol) solutions, *Polymer*, **17**, 685–689, doi: 10.1016/0032–3861(76)90208–1.

Sage, I. (2011) Thermochromic liquid crystals, *Liquid Crystals*, **38**, 1551–1561.

Scarpa, J. S., Mueller, D. D. and Klotz, I. M. (1967) Slow hydrogen-deuterium exchange in a non-alpha-helical polyamide, *J Am Chem Soc*, **89**, 6024–6030, doi: 10.1021/ja01000a006.

Schäfer-Soenen, R., Moerkerke, R., Berghmans, H., Koningsveld, R., Dusek, K. and Solc, K. (1997) Zero and off-zero critical concentrations in systems containing polydisperse polymers with very high molar masses. 2. The system water-poly(vinyl methyl ether), *Macromolecules*, **30**, 410–416, doi: 10.1021/ma960114o.

Schild, H. G. (1992) Poly(*N*-isopropylacrylamide): Experiment, theory and application, *Prog Polym Sci*, **17**, 163–249, doi: 10.1016/0079–6700(92)90023-R.

Schmaljohann, D. (2006) Thermo- and pH-responsive polymers in drug delivery, *Adv Drug Delivery Rev*, **58**, 1655–1670, doi::10.1016/j.addr.2006.09.020.

Schröder, G. (2000) Poly(vinyl ethers). In: *Ullmann's Encyclopedia of Industrial Chemistry*, Weinheim, Wiley-VCH.

Schultz, A. R. and Flory, P. J. (1952) Phase equilibria in polymer-solvent systems, *J Am Chem Soc*, **74**, 4760–4767, doi: 10.1021/ja01139a010.

Schulz, D. N., Peiffer, D. G., Agarwal, P. K., Larabee, J., Kaladas, J. J., Soni, L., Handwerker, B. and Garner, R. T. (1986) Phase behaviour and solution properties of sulphobetaine polymers, *Polymer*, **27**, 1734–1742, doi: 10.1016/0032–3861(86)90269–7.

Seuring, J. and Agarwal, S. (2010) Non-ionic homo- and copolymers with H-donor and H-acceptor units with an UCST in water, *Macromol Chem Phys*, **211**, 2109–2117, doi: 10.1002/macp.201000147.

Seuring, J. and Agarwal, S. (2012a) Polymers with upper critical solution temperature in aqueous solution, *Macromol Rapid Commun*, **33**, 1898–1920, doi: 10.1002/marc.201200433.

Seuring, J. and Agarwal, S. (2012b) First example of a universal and cost-effective approach: Polymers with tunable upper critical solution temperature in water and electrolyte solution, *Macromolecules*, **45**, 3910–3918, doi: 10.1021/ma300355k.

Shimada, N., Ino, H., Maie, K., Nakayama, M., Kano, A. and Maruyama, A. (2011) Ureido-derivatized polymers based on both poly(allylurea) and poly(*L*-citrulline) exhibit UCST-type phase transition behavior under physiologically relevant conditions, *Biomacromolecules*, **12**, 3418–3422, doi: 10.1021/bm2010752.

Skrabania, K., Kristen, J., Laschewsky, A., Akdemir, O., Hoth, A. and Lutz, J.-F. (2007) Design, synthesis, and aqueous aggregation behavior of nonionic single and multiple thermoresponsive polymers, *Langmuir*, **23**, 84–93, doi: 10.1021/la061509w.

Solomon, O. F., Corciovei, M., Ciuta, I and Boghina, C. (1968) Properties of solutions of poly-*N*-vinylcaprolactam, *J Appl Polym Sci*, **12**, 1835–1842.

Steinhauer, W., Hoogenboom, R., Keul, H. and Moeller, M. (2010) Copolymerization of 2-hydroxyethyl acrylate and 2-methoxyethyl acrylate via RAFT: Kinetics and thermoresponsive properties, *Macromolecules*, **43**, 7041–7047, doi: 10.1021/ma101122b.

Stryer, L. (1999) *Biochemistry*, New York, Freeman.

Urry, D. W. (1984) Protein elasticity based on conformations of sequential polypeptides: the biological elastic fiber, *J Protein Chem*, **3**, 403–436, doi: 10.1007/BF01025061.

Uyama, H. and Kobayashi, S. (1992) A novel thermo-sensitive polymer. Poly(2-*iso*-propyl-2-oxazoline), *Chem Lett*, 1643–1646, doi: 10.1246/cl.1992.1643.

van Assche, G., van Mele, B., Li, T. and Nies, E. (2011) Adjacent UCST phase behavior in aqueous solutions of poly(vinyl methyl ether): Detection of a narrow

low temperature UCST in the lower concentration range, *Macromolecules*, **44**, 993–998, doi: 10.1021/ma102572s.

van Durme, K., van Assche, G. and van Mele, B. (2004) Kinetics of demixing and remixing in poly(*N*-isopropylacrylamide)/water studied by modulated temperature DSC, *Macromolecules*, **37**, 9596–9605, doi: 10.1021/ma048472b.

van Vlierberghe, S., Dubruel, P. and Schacht, E. (2011) Biopolymer-based hydrogels as scaffolds for tissue engineering applications: A review, *Biomacromolecules*, **12**, 1387–1408, doi: 10.1021/bm200083n.

Vihola, H., Laukkanen, A., Valtola, A., Tenhu, H. and Hirvonen, J. (2005) Cytotoxicity of thermosensitive polymers poly(*N*-isopropylacrylamide), poly(*N*-vinylcaprolactam) and amphiphilically modified poly(*N*-vinylcaprolactam), *Biomaterials*, **26**, 3055–3064, doi: 10.1016/j.biomaterials.2004.09.008.

Ward, M. A. and Theoni, T. K. (2011) Thermoresponsive polymers for biomedical applications, *Polymers*, **3**, 1215–1242, doi: 10.3390/polym3031215.

Weber, C., Hoogenboom, R. and Schubert, U. S. (2012) Temperature responsive biocompatible polymers based on poly(ethylene oxide) and poly(2-oxazoline)s, *Prog Polym Sci*, **37**, 686–714, doi: 10.1016/j.progpolymsci.2011.10.002.

Weber, C., Rogers, S., Vollrath, A., Hoeppener, S., Rudolph, T., Fritz, N., Hoogenboom, R. and Schubert, U. S. (2013) Aqueous solution behavior of comb-shaped poly(2-ethyl-2-oxazoline), *J Polym Sci Part A Polym Chem*, **51**, 139–148, doi: 10.1002/pola.26332.

Weiss, R. A. and Ober, C. K. (1990) Liquid-crystalline polymers, *ACS Symp Ser*, **435**, entire volume.

Xie, T. (2010) Tunable polymer multi-shape memory effect, *Nature*, **464**, 267–270, doi: 10.1038/nature08863.

Yan, N., Zhang, J., Yuan, Y., Chen, G.-T., Dyson, P. J., Li, Z.-C. and Kou, Y. (2010) Thermoresponsive polymers based on poly-vinylpyrrolidone: applications in nanoparticle catalysis, *Chem Commun*, **46**, 1631–1633, doi: 10.1039/b923290g.

Yang, H., Zhu, H., Hendrix, M. M. R. M., Lousberg, N. J. H. G. M., de With, G., Esteves, A. C. C. and Xin, J. H. (2013) Temperature-triggered collection and release of water from fogs by a sponge-like cotton fabric, *Adv Mater*, **25**, 1150–1154, doi: 10.1002/adma.201204278.

Yuan, Y.-Y., Du, J.-Z. and Wang, J. (2012) Two consecutive click reactions as a general route to functional cyclic polyesters, *Chem Commun*, **48**, 570–572, doi: 10.1039/c1cc16065f.

3

pH-responsive polymers: properties, synthesis and applications

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Abstract: This chapter summarizes the properties and most representative applications of pH-responsive polymers in the biomedical field. The most common methodologies to synthesize pH-responsive polymers such as emulsion polymerization, group transfer polymerization, atom transfer radical polymerization and reversible addition-fragmentation chain transfer polymerization are described. This chapter also discusses the most important applications of pH-responsive polymers in drug and gene delivery and the use of these systems as biosensors, taking into account the chemical and physical properties of these smart polymer systems.

Key words: pH-responsive polymer, drug and gene delivery, hydrogels and nanoparticles carriers, control radical polymerization.

3.1 Introduction

pH-sensitive polymers can be defined as polyelectrolytes that include in their structure weak acidic or basic groups that either accept or release protons in response to a change in the environmental pH. The acidic or basic groups of these polyelectrolytes can be ionized just like acidic or basic groups of monoacids or monobases; however, complete ionization of these systems is more difficult due to electrostatic effects exerted by other adjacent ionized groups. This makes the apparent dissociation constant (K_a) different from that of the corresponding monoacid or monobase. The physical properties, such as chain conformation, configuration, solubility and volume of pH-responsive polymers, could be tailored by manipulating the charges along the polymer backbone or electrolyte concentrations, resulting in electrostatic repulsion forces that create an increase in the hydrodynamic volume of the polymer. This transition between tightly coiled and expanded state is influenced by any condition that modifies electrostatic repulsion,

such as pH, ionic strength and type of counterions. The transition from collapsed state to expanded state is explained by changes in the osmotic pressure exerted by mobile counterions neutralizing the network charges (Dai *et al*., 2008).

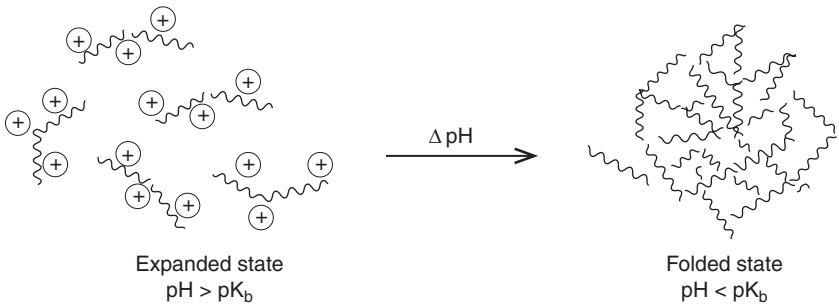
The pH range where the reversible phase transition happens can generally be modulated in two ways: selecting the ionizable moiety with a pK_a matching the desired pH range (selecting between polyacid or polybase) and incorporating hydrophobic moieties into the polymer backbone (selectively control their nature, amount and distribution) (Na *et al*., 2004). When ionizable groups become neutral – non-ionized – and electrostatic repulsion forces disappear within the polymer network, hydrophobic interactions dominate. The introduction of a more hydrophobic moiety can offer a more compact conformation in the uncharged state and a more accused phase transition (Sukhishvili, 2005).

pH-responsive polymers can be synthesized through conventional or controlled radical polymerization techniques (Gregory and Stenzel, 2012a; Mu *et al*., 2011). Emulsion polymerization is among the most popular synthetic route for preparing vinyl-based, pH-responsive particulate systems, especially microgel systems (Chuang *et al*., 2009; Gao *et al*., 2009). A variety of synthetic methodologies are described in this chapter, and some of their salient features are highlighted.

Several polymeric structures, such as homopolymers (Tian *et al*., 2012), block copolymers (Yao *et al*., 2011; Yuan *et al*., 2011), microgels, hydrogels (HGs) (Ramírez *et al*., 2011; Wu *et al*., 2011), micro- and nanoparticles (NP) (Yang and Liu, 2013) and polymer brushes (Lee *et al*., 2010) will be described in this chapter, where important characteristics that govern their behavior in solutions are explained. The 'intelligent' properties of these pH-responsive polymeric systems are attractive for applications in life sciences and the chemical industry, providing potential applications, such as in controlled drug delivery, personal care, industrial coatings, oil exploration, water remediation, etc. (Brun-Graeppi *et al*., 2011; Calderón *et al*., 2010; Ho *et al*., 2011; Luzinov *et al*., 2004; Wever *et al*., 2011; Zhao *et al*., 2011).

3.2 Key types and properties of pH-responsive polymers

In this section, the properties, characteristics and uses for pH-responsive polymer systems are described. The advancement in material science has led to the design of a variety of sensitive materials, which are used for development of new polymeric systems that can respond to biological stimuli. There is a highly promising role of pH-responsive polymer systems for drug and gene delivery in the future, as it is described in the following sections of this chapter.



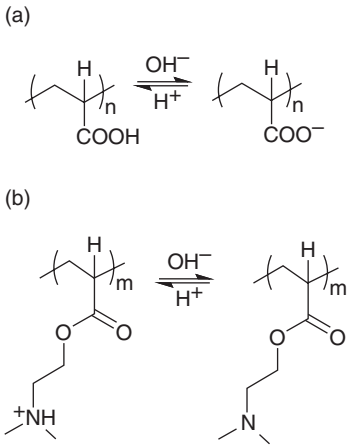
3.1 Polybasic states depending of the ionization of the ionic chain groups of the pH-responsive polymer.

3.2.1 Definition and general properties

A polyelectrolyte is a macromolecule that can dissociate to give polymeric ions when dissolved in water or other ionizing solvents. Because of the repulsion between charges on the polymer chain, the system expands when it is ionized in a suitable solvent. However, if the solvent prevents ionization of the polyelectrolyte, the dissolved chain remains in a compact, folded state (Fig. 3.1). If the polyelectrolyte chains are hydrophobic when unionized in a poor solvent, they collapse into globules and precipitate from solution. The interplay between hydrophobic surface energy and electrostatic repulsion between charges dictates the behavior of the polyelectrolyte. Since the degree of ionization of a weak polyelectrolyte is controlled by pH and the ionic composition of the aqueous medium, pH-sensitive polymers dramatically change conformation in response to minute changes in the pH of the aqueous environment.

Medium pH controls polybase conformation changing from an expanded state to a folded state depending on the ionization degree of the pH-responsive polymer.

pH-responsive polymers contain either weakly acidic (e.g., carboxylic acid) or basic (e.g., ammonia) groups, these either release protons or accept free protons, respectively, in response to environmental pH. Under certain pH conditions the functional groups presented along the backbone and side chains of the polymer undergo ionization that leads to a conformational change in the polymer resulting in its swelling or dissolution. For example, poly(acrylic acid) (PAA) has a dissociation constant (pK$_a$ 4.25) and above this pH the carboxylic group becomes ionized (Fig. 3.2a). This leads to electrostatic repulsion between the chains that can then associate with water to cause swelling. In addition, other polymers show an inverse behavior. The cationic polyelectrolyte poly(*N,N*-dimethylaminoethyl methacrylate)



3.2 Structures of pH-responsive polyelectrolytes (a) poly(acrylic acid) (PAA) and (b) poly(*N,N*-dimethylaminoethyl methacrylate) (PDMAEMA).

(PDMAEMA) shows ionized behavior at low pH values (Fig. 3.2b). Charge status in these materials is readily reversed by returning the pH of the solution; thus the switching behavior of pH-responsive smart materials is also reversible.

All pH-sensitive polymers contain pendant acidic or basic groups that either accept or donate protons in response to the environmental pH. Polyacid polymers will be unswollen at low pH, since the acidic groups will be protonated and unionized. When increasing the pH, a negatively charged polymer will swell. The opposite behavior is found in polybasic polymers, since the ionization of the basic groups will increase when decreasing the pH. Typical examples of pH-sensitive polymers with anionic groups are poly(carboxylic acids) as PAA or poly(methacrylic acid) (PMA) and polysulfonamides (Kang and Bae, 2002) (derivatives of p-aminobenzene sulfonamide). These weak polyacids present a pK_a that narrowly varies from 3 to 11, depending on the electron removing nature of the substituent on the nitrogen. At low pH, carboxyl groups are protonated and hydrophobic interactions dominate, leading to volume withdrawal of the polymer that contains the carboxyl groups. At high pH, carboxyl groups dissociate into carboxylate ions, resulting in high charge density in the polymer, causing it to swell. The chain configuration of weak polyacid is a function of the pK_a of the polymer.

An opposite behavior is shown by cationic polyelectrolytes, for example poly(*N,N*-dialkyl aminoethyl methacrylates), poly(L-lysine) (PLL), poly(ethylenimine) (PEI) and chitosan. These polyelectrolytes are acid-swellable groups, in contrast to the alkali-swellable carboxyl group. Under acidic environments, the polybasic groups are protonated, increasing the

internal charge repulsions between neighboring protonated polybasic groups. Charge repulsion leads to an expansion in the overall dimensions of the polymer containing the groups. At higher pH values, the groups become less ionized, the charge repulsion is reduced and the polymer–polymer interactions increase, leading to a decrease of the overall hydrodynamic diameter of the polymer.

These characteristics are used, for example, to obtain pH-responsive HGs which are widely used as carriers in drug delivery systems (Zhao *et al.*, 2008). Swelling of a hydrogel increases as the external pH increases in the case of weakly acidic (anionic) groups, but decreases if the polymer contains weakly basic (cationic) groups. When the ionic strength of the solution is increased, the hydrogel can exchange ions with the solution. In that way, the hydrogel keeps charge neutrality, and the concentration of free counter ions inside the hydrogel increases. An osmotic pressure difference between the hydrogel and the solution appears and causes the gel to swell. If ionic strength is equal to or higher than 1–10 M, the hydrogel will shrink. This is due to the decreasing osmotic pressure difference between the gel and the solution. The solution now has an osmotic pressure in the range of the osmotic pressure inside the gel (Guvendiren *et al.*, 2009).

Most anionic pH-sensitive polymers are based on PAA or its derivatives. These systems usually contain moieties anionically charged at a pH above their pK_a and can attract positively charged therapeutic agents (Palasis, US6506408 B1 Patent), (Dash and Cudworth Ii, 1998). Peppas and co-workers prepared HGs of poly(methacrylic acid-g-ethylene glycol) P(MAA-g-EG) loaded with insulin exhibited unique pH-responsive characteristics whereby interpolymer complexes formed in acidic media and dissociated in neutral/basic environments (Serra *et al.*, 2006). Consequently, insulin release from the gel was significantly retarded in acidic media while rapid release occurred under neutral/basic conditions.

The interaction between pH-sensitive polymers and its biological environment ultimately governs how biological processes proceed on these materials, for example, biomolecule adsorption/desorption, cellular interaction. By controlling the surface physical and chemical properties of materials the interfacial characteristics can be altered to dictate these interactions. The versatility with which the surface characteristics can be manipulated and switched using external stimuli means pH-sensitive polymers have received much interest for the potential to alter biological interactions/functions. Many biological mechanisms are strongly affected by the levels of charge in ionic strength required to switch such materials. The adjustment in pH alters the ionic interaction, hydrogen bonding and hydrophobic interaction, resulting in a reversible microphase separation or self-organization phenomenon. For example, since the extracellular pH of most tumors is acidic (pH 5.8–7.2), smart polymeric nano-devices can be designed for anti-cancer drug

delivery, where the release of drugs can be triggered by manipulating pH. The pH triggering could be done by incorporating a pH-responsive moiety to the polymer structure, destabilizing a self-assembled polymeric aggregate or by chemical conjugation of pH-liable linkage between polymers and drugs. These strategies are particularly useful in targeted drug delivery (Gil and Hudson, 2004).

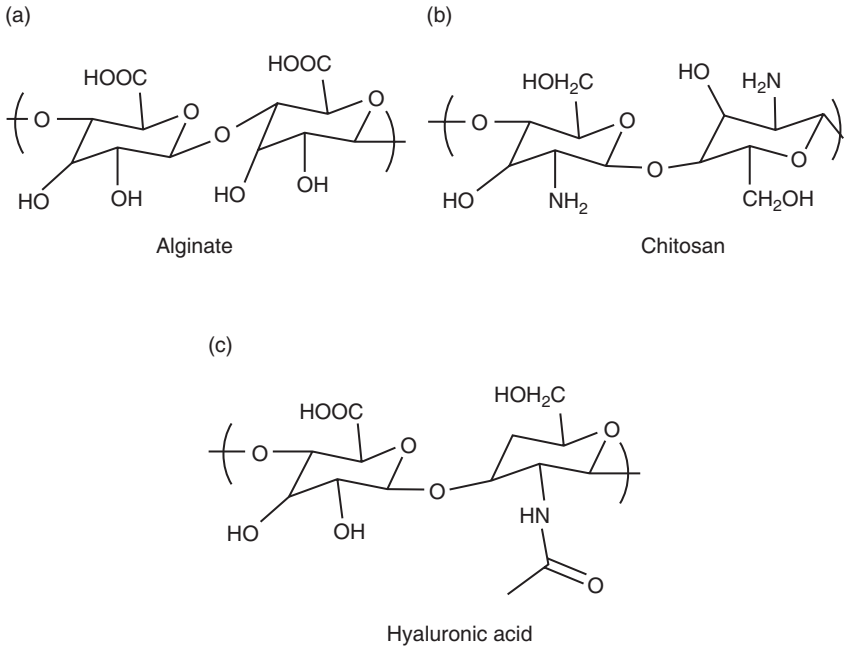
3.2.2 Natural polymers

Although a number of synthetic biodegradable polymers have been developed for biomedical applications, the use of natural biodegradable polymers remains attractive because of their abundance in nature, good biocompatibility and ability to be readily modified by simple chemistry (Sonia and Sharma, 2012). Biopolymers are polymers which form a part of living cells, proteins, carbohydrates, nucleic acid and, therefore, they are widely used in pharmaceutical formulations and, in several cases, they play a fundamental role in determining the release rate from the dosage form. For that, biopolymers are promising materials in the delivery of protein drugs due to their compatibility, degradation behavior and nontoxic nature on administration. On suitable chemical modification, these polymers can provide better materials for drug delivery systems.

The salient feature of functional biopolymers is their all-or-none linear response to external stimuli. Small changes happen in response to varying parameters until the critical point is reached, when the transition occurs in the narrow range of the parameter variation and, after the transition is completed, there is no significant further response of the system. Despite the weakness of each particular interaction taking place in a separate monomer unit, these interactions when summed through hundreds and thousands of monomer units could provide significant driving forces for the processes occurring in such systems. The use of biopolymers like dextran, chitosan, alginate, hyaluronic acid (HA) and pullulan is very common in drug delivery systems. In this chapter the three most important biopolymers used as pH-responsive polymers to create novel species and invent new processes for drug delivery applications will be discussed (Fig. 3.3).

Alginate

Alginate is a non-branched, high-molecular weight binary copolymer of (1–4) glycosidically linked β-D-mannuronic acid and α-L-glucuronic acid monomers (Fig. 3.3a). The high acid content allows alginic acid to undergo spontaneous and mild gelling in the presence of divalent cations, such as calcium ions. These mild gelling properties are pH dependent and allow the encapsulation of various molecules or even cells within alginate gels with



3.3 Structure of (a) alginate (b) chitosan (c) hyaluronic acid, pH-responsive biopolymers used in drug delivery systems.

minimal negative impact. Further, the carboxylic acid groups of alginic acid are highly reactive and can be appropriately modified for various applications. Alginate has been extensively investigated as a drug delivery device wherein the rate of drug release can be varied by the drug polymer interactions varying as well as by chemical immobilization of the drug to the polymer backbone using the reactive carboxylate groups (Gong *et al.*, 2011). Hydrophobically modified alginates are also used for drug delivery applications. The encapsulation of proteins and bioactive factors within ionically cross-linked alginate gels are known to greatly enhance their efficiency and targetability and, as a result, extensive investigation has been undertaken to develop protein delivery systems based on alginate gels (Yang and Liu, 2013).

Alginate surface is negatively charged; therefore, when positively charged polymers are added to the alginate solution, they can form a polycation–polyanion complex, which will enhance the overall stability of the microcapsules (Joshi *et al.*, 2011). A disadvantage of using alginate-based gels, apart from their poor degradability, is poor cell adhesion on alginate gel. Several natural polymers such as chitosan have been combined with sodium alginate in order to increase the encapsulation efficiency and hence the protein

release profiles. At low pH (gastric environment), alginate shrinks and the encapsulated drug is not released. For example, insulin could be loaded by liposomes and this lipoinsulin system can be entrapped in an alginate system. The aqueous interior of the liposome will preserve the structure and conformation of insulin, while the lipid exterior may help improve absorption across biological barriers. Oral administration of lipoinsulin-loaded alginate-chitosan capsules was found to reduce blood glucose level in diabetic rats (El-Sherbiny, 2010; Silva *et al*., 2006).

Chitosan

Chitosan is the only cationic polysaccharide of natural origin, obtained by the alkaline, partial deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans and cell walls of fungi. It is a versatile biopolymer of the aminoglucopyran family, widely used for various biomedical and pharmaceutical applications including drug delivery. Chitosan is a cationic amino polysaccharide (pK_a 6.5) copolymer of glucosamine and *N*-acetylglucosamine (Fig. 3.3b). Chitosan is a biodegradable, biocompatible, positively charged nontoxic mucoadhesive biopolymer. Since chitosan contains primary amino groups in the main backbone that make the surfaces positively charged in biological fluids, biodegradable nano/microparticles can be readily prepared by treating chitosan with a variety of biocompatible polyanionic substances such as sulfate, citrate and tripolyphosphate. These unique features of chitosan have stimulated the development of a wide range of biological agent delivery systems. Chitosan has been reported to enhance drug permeation across the intestinal, nasal and buccal mucosa (Şenel *et al*., 2000). Chitosan microspheres have arisen as a promising candidate in oral or other mucosal administration for improving the transport of biomacromolecules such as peptides, proteins, oligonucleotides and plasmids across biological surfaces.

Chitosan-based scaffolds possess some special properties for use in tissue engineering. First, chitosan can be formed as interconnected-porous structures by freezing and lyophilizing a chitosan solution (Kirsebom *et al*., 2007) or by processes such as an 'internal bubbling process' where $CaCO_3$ is added to chitosan solutions to generate chitosan–$CaCO_3$ gels in specific shapes by using suitable molds (Meldrum, 2003). The interconnected-porous structure is very important, so that numerous cells can be seeded, migrate into the inside, increase the cell number and should be supplied by sufficient amounts of nutrient. Second, the cationic nature of chitosan also allows for pH-dependent electrostatic interactions with anionic glycosaminoglycans (GAG) and proteoglycans distributed widely throughout the body and other negatively charged species (George and Abraham, 2006). This property is one of the important elements for tissue engineering applications because

numbers of cytokines/growth factors are known to be bound and modulated by GAG including heparin and heparan sulfate. A scaffold incorporating a chitosan–GAG complex may serve as a means of retaining and concentrating desirable factors secreted by colonizing cells. Moreover, Nishikawa *et al*. (2000) reported that chitosan, structurally similar to GAG, consisting of long-chain, unbranched, repeating disaccharide units, was able to play a key role in modulating cell morphology, differentiation and function.

Chitosan has been shown to degrade *in vivo*, which is mainly by enzymatic hydrolysis. The degradability of a scaffold plays a crucial role in the long-term performance of tissue-engineered cell/material constructs because it affect many cellular process, including cell growth, tissue regeneration and host response. If a scaffold is used for tissue engineering of the skeletal system, degradation of the scaffold biomaterial should be relatively slow, as it has to maintain the mechanical strength until tissue regeneration is almost completed.

One of the properties of chitosan is that it confers considerable antibacterial activity against a broad spectrum of bacteria. The interaction between the positively charged chitosan and negatively charged microbial cell wall leads to the leakage of intracellular constituents. The binding of chitosan with DNA and inhibition of mRNA synthesis occurs via the penetration of chitosan into the nuclei of the microorganisms and interference with the synthesis of mRNA and proteins. Due to this antibacterial property, chitosan has been blended with other polymers (Hu *et al*., 2003).

The field of wound healing has been another major emphasis in chitosan-based medical applications research (Kim *et al*., 2008). A number of researchers have examined the host tissue response to various chitosan-based implants. In general, these materials have been found to evoke a minimal foreign body reaction, with little or no fibrous encapsulation. It observed the typical course of healing with formation of normal granulation tissue, often with accelerated angiogenesis. Actually, chitosan possesses the properties favorable for promoting rapid dermal regeneration and accelerate wound healing suitable for applications extending from simple wound coverings to sophisticated artificial skin matrices (Alemdaroğlu *et al*., 2006).

Hyaluronic acid

Hyaluronic acid has attracted particular attention due to its abundant existence in living organisms and the human body. HA is a linear anionic polysaccharide made of a repeated disaccharide of (1–3)- and (1–4)-linked β-D-glucuronic acid and *N*-acetyl β-D-glucosamine monomer (Fig. 3.3c). It is the only non-sulfated GAG and plays an important role in the organization and stabilization of the extracellular matrix (ECM), cell proliferation and differentiation (Ekici *et al*., 2011). HA is also involved in the

morphogenesis, in inflammation and wound repair (Pérez *et al*., 2013; Puppi *et al*., 2010; Zhang *et al*., 2005). Due to its importance *in vivo* and its potential in tissue engineering, several strategies have been developed to prepare HA HGs including disulfide cross-linking and photocross-linking (Collins and Birkinshaw, 2013).

HA as biomaterial presents a variety of functions, including maintenance of the overall conformation of matrix surrounding cells and the creation of cell-free spaces upon hydration. HA can also activate cell surface receptors that influence intracellular signaling cascades affecting cell behaviors such as growth, migration and differentiation. In the nervous system, HA functions, in conjunction with a number of HA-binding proteoglycans as well as with collagen, have been implicated in regulating glial cell and neuron migration, neurite outgrowth and axon pathfinding (Bovolenta and Fernaud-Espinosa, 2000). HA has been combined with alginates and PLL to develop scaffolds for a variety of tissue engineering applications including nerve regeneration. Tan *et al*. (2012) reported on the development of a new class of biocompatible and biodegradable composite HGs derived from water-soluble chitosan and oxidized HA or alginate without the addition of a chemical cross-linking agent. The gelation was attributed to the Schiff base reaction between amino and aldehyde groups of polysaccharide derivatives including *N*-succinyl-chitosan and aldehyde-modified HA.

HA in combination with collagen has been used to form semi-interpenetrating networks (SIPNs) and endothelial cell attachment was realized within microfluidic channels aiming at blood vessel formation. These SIPNs were suitable for enabling fibroblast and chrondrocyte encapsulation and subsequent proliferation (Ji *et al*., 2011).

3.2.3 Dual responsive materials

In addition to the single response polymer it is also possible to design and engineer materials that respond simultaneously to a combination of stimuli (e.g., temperature, pH, magnetic fields). By combining two properties this creates a polymer that is more specific and controllable. Temperature and pH-responsive polymers can be obtained by the copolymerization of monomers bearing hydrophobic and ionizable functional groups, monomers which included a magnetic group, or by the polymerization of dual responsive monomers.

pH–thermo-responsive polymers

Cell or protein smart delivery systems are desirable as they allow precise application to a local site. For optimal design the material should be a solution at room temperature and gel within the body at physiological temperature and

pH. Polymers with both temperature and pH-responsive moieties have been created keeping this in mind. Lee *et al*. (2010) synthesized a pH-sensitive block copolymer via atom transfer radical polymerization (ATRP) of sulfamethazine methacrylate (SM) monomer and amphiphilic diblock copolymers by the ring-opening polymerization of D,L-lactide/ε-caprolactone (PCLA), and their sol–gel phase transition was investigated. SM, which is a derivative of sulfonamide, was used as a pH-responsive moiety, while PCLA–PEG–PCLA was used as a biodegradable, as well as a temperature-sensitive, amphiphilic triblock copolymer. The pentablock copolymer, SM–PCLA–PEG–PCLA–SM, was synthesized using Br–PCLA–PEG–PCLA-Br as an ATRP macroinitiator (Dayananda *et al*., 2007). Yuan *et al*. (2009) synthesized a hydrogel of ethyl 2-acetyl-3-oxo-4-pentenoate (4-acetyl acryloyl ethyl acetate (AAEA)) and acrylic acid (AA) using azoisobutyronitrile (AIBN) as initiator and *N,N′*-methylene bis(acrylamide) as cross-linking agent. The volume phase transition temperature (VPTT) of the HGs showed that the volume phase transition (VPT) of the HGs ranged from 50.3°C to 61.8°C and increased significantly with the increasing dosage of AA. These series of HGs exhibited good thermo-sensitivity. When the temperature was higher than the critical temperature, the HGs shrank significantly. Moreover, the critical temperature of the HGs increased significantly with the increasing dosage of AA. The pH sensitivity of the HGs indicated that the HGs exhibited a discontinuous volume change at around pH = 9.5 (Yuan *et al*., 2009).

This technology has been expanded to prepare intelligent nanocapsules with temperature-responsive cross-linked shells and pH-responsive brushes on their inner walls. These nanocapsules have been prepared by the surface-initiated atom transfer radical polymerization (SI-ATRP) technique with silica NP as the sacrificial templates. The two-step, sequential SI-ATRP procedure provided the poly(tert-butyl acrylate) (PtBA) brushes on the inner walls of the temperature-responsive cross-linked poly(*N*-isopropylacrylamide) (PNIPAA) shells. Then the ester groups in the nanocapsules were transformed chemically into carboxyl groups after etching the silica templates with HF (Mu and Liu, 2012).

Pathologies that result in an acidic environment are ideal candidates for treatment using dual responsive smart materials. Garbern *et al*. (2011) created a pH- and temperature-responsive hydrogel that had the ability to deliver bioactive molecules to a site of cardiac infarct in rat models. This system could improve therapeutic angiogenesis methods by providing spatial-temporal control of angiogenic growth factor delivery. The scaffold, developed from p(NIPAA-co-PAA-co-butyl acrylate (BA)) was liquid at pH 7.4 and 37°C but formed a physical gel at pH 6.8 at the same temperature. When polymer doped with basic fibroblast growth factor (bFGF) was administered to a site of ischemic myocardial, spatial-temporal control over delivery was observed compared to administration in saline, and treatment over 28 days led to increased angiogenesis and regional blood

flow. By responding to local changes in pH and temperature in an animal model of ischemia, this hydrogel system provided sustained, local delivery of bFGF, improved angiogenesis and achieved therapeutic effects in regional blood flow and cardiac function (Garbern *et al*., 2011).

pH–magneto-responsive polymers

The nature of the chemical composition of magnetic nanoparticles (MNPs) gives rise to a magneto-responsive behavior. Consequently, as soon as a MNP becomes functionalized with pH- or temperature-responsive polymers, it becomes a dually responsive material. Recently, a number of authors have presented studies using responsive-coated MNPs for the treatment of cancer (Chan *et al*., 2013). Wang *et al*. (2004) attempted to create an 'all-in-one' particle for the treatment of cancer. This involved a superparamagnetic iron oxide core surrounded by a mesoporous silica shell and folic acid to increase particle uptake by cancerous cells. The anti-cancer drug Doxorubicin (DOX) was loaded into the pores and ferrocene was linked to the pores to prevent passive release of the drug. Under physiological levels of pH there was a limited release of DOX; however, in more acidic conditions, pH 6–4.5 there was a marked increase of drug release. The ferrocene Schiff base linker acted as the pH-responsive material to control drug release under acidic conditions similar to those found in a tumor microenvironment (Zhu *et al*., 2012). Similarly, Lin *et al*. (2010) reported a controlled release system based on upconversion luminescent microspheres of $NaYF_4$:Yb^{3+}/Er^{3+} coated with the smart hydrogel poly(NIPAM-*co*-MAA) composing its shell. They exploited the hybrid microspheres as carriers for DOX hydrochloride due to its stimuli-responsive property as well as good biocompatibility. It is found that the drug release behavior is pH-triggered and thermally sensitive. Changing the pH to mildly acidic conditions at physiological temperature deforms the structure of the shell, causing the release of a large amount of DOX from the microspheres. The drug-loaded microspheres exhibit an obvious cytotoxic effect on SKOV3 ovarian cancer cells. These pH-induced, thermally controlled drug release systems have potential to be used for *in vivo* bioimaging and cancer therapy by the pH of the microenvironment changing from 7.4 (normal physiological environment) to acidic microenvironments (such as endosome and lysosome compartments) owing to endocytosis (Dai *et al*., 2012).

3.3 Synthesis of pH-responsive polymers

In this section the most common methodologies used to synthesize pH-responsive polymers are described. Summarizing, they are the emulsion polymerization (micro- and mini-emulsion), controlled living radical polymerization techniques (atom transfer radical polymerization (ATRP),

reversible addition-fragmentation chain transfer (RAFT), group transfer polymerization (GTP)) and ionic polymerization. The pH-responsive polymers prepared by these techniques show well-defined properties and, depending on the methodology used to get it, the structure of the polymer chains can be linear homo or copolymers, amphiphilic block copolymers that form micelles, microgels, HGs, micro- or nanoparticles, dendritic polymers and polymer brushes at interfaces.

3.4 Different methodologies for the preparation of pH-responsive polymers

In the next section, the most innovated and frequently used methods for the preparation of pH-responsive polymers are described.

3.4.1 Emulsion polymerization

Emulsion polymerization is among the most popular synthetic routes to prepare vinyl-based pH-responsive polymers, especially microgel systems (Rao and Geckeler, 2011). This technique employs a radical chain polymerization methodology to form latexes of narrow particle size distributions. The emulsion polymerization systems are commonly composed of monomer(s), water, water-soluble initiator and surfactant (emulsifier). Colloidal stabilizers may be electrostatic, steric or electrosteric, or display both stabilizing mechanisms. When phase separation occurs, the formation of solid particles takes place before or after the termination of the polymerization reaction.

One of the disadvantages of this technique is the use of surfactants which may need to be removed at the end of the polymerization reaction, but it is not always easy to carry out. The removal of surfactant, either by dialysis or desorption, may lead to coagulation or flocculation of the latex. An alternative process is the surfactant-free emulsion polymerization characterized by the absence of added emulsifier (Rao and Geckeler, 2011). This kind of emulsion polymerization can be used to prepare well-defined core-shell NPs. Tan *et al*. (2005) prepared core-shell NPs containing a poly(methyl methacrylate) (PMMA) core and a P(MMA-co-EA) shell. The PMMA core was first synthesized through conventionally seeded emulsion polymerization, and the secondly pre-emulsified monomers and small amounts of initiator were introduced slowly under monomer-starved feeding conditions to grow the pH-responsive shell layer. Tam *et al*. (2006) described other systems composed of poly(methyl acrylamide) (PMAA) and PDMAEMA chains. In contrast to the PMAA or PAA system, such latex is swellable at low pH due to the protonation of amino segments. Further, the emulsion polymerization has also been used to prepare core-shell hybrid materials

containing an inorganic core. Hollow nanocages could be produced by etching the metallic core after cross-linking of the water-swellable shell layer (Zhang *et al*., 2008).

Mini-emulsion polymerization

A typical formulation used in mini-emulsion polymerization consists of water, monomer mixture, co-stabilizer, surfactant and initiator. The key difference between emulsion polymerization and mini-emulsion polymerization is the utilization of a low molecular mass compound as the co-stabilizer and also the use of a high-shear device (ultrasound, etc.). Mini-emulsions are critically stabilized, require a high-shear to reach a steady state and have an interfacial tension much greater than zero (Koul *et al*., 2011; Winkelmann and Schuchmann, 2011).

Versatile particles have been developed with various co-stabilizers and initiator combinations (Baruch-Sharon and Margel, 2010). These combinations have a predominant influence on the formation and nature of the NPs (Jiang *et al*., 2010). PAA NPs were synthesized by Kriwet *et al*. (Kriwet *et al*., 1998) using a co-emulsifier system consisting of a mixture of Span 80 and Tween 80. The polymerization was initiated by free radicals and the particle size was dependent on the type of radical initiator used. Using water-soluble initiators, such as ammonium persulfate (APS), microparticles were obtained; however, NPs were generated almost exclusively with a diameter between 80 and 150 nm when lipophilic radical initiators, such as AIBN, were used.

Micro-emulsion polymerization

Micro-emulsion polymerization is a new and effective approach for preparing nanosized polymer particles and has attracted significant attention. Emulsion and micro-emulsion polymerization differ in the kinetics of the polymerization. Emulsion polymerization exhibits three reaction rate intervals, whereas only two are detected in micro-emulsion polymerization. Both particle size and the average number of chains per particle are considerably smaller in micro-emulsion polymerization (Schork *et al*., 2005).

In micro-emulsion polymerization, an initiator, typically water-soluble, is added to the aqueous phase of a thermodynamically stable micro-emulsion containing swollen micelles. The polymerization starts from this thermodynamically stable, spontaneously formed state and relies on high quantities of surfactant systems, which possess an interfacial tension at the oil/water interface close to zero. Furthermore, the particles are completely covered with surfactant because of the utilization of a high amount of surfactant. Initially, polymer chains are formed only in

Table 3.1 Summary of the most important properties of conventional emulsion, mini-emulsion and micro-emulsion

Emulsion type	Conventional emulsion	Mini-emulsion	Micro-emulsion
Particle size range	50–500 nm	50–500 nm	10–100 nm
Duration of stability	Seconds to hours	Hours to months	Indefinitely
Diffusional stabilization	Kinetic	Kinetic	Thermodynamic
Nucleation mechanism	Micellar, homogeneous	Droplet	Droplet
Emulsifier concentration	Moderate	Moderate	High
Co-stabilizer type	None	Hexadecane, cetyl alcohol	Hexanol, pentanol
Homogenization method	None	Mechanical or ultrasonic	None

some droplets, as the initiation cannot be reached simultaneously in all microdroplets. Later, the osmotic and elastic influence of the chains destabilize the fragile micro-emulsions and typically lead to an increase in the particle size, the formation of empty micelles and secondary nucleation (Wang *et al.*, 2004). In the final product very small latexes, 5–50 nm in size, coexist with a majority of empty micelles. The types and concentration of initiator, surfactant and monomer and the reaction temperature are some of the critical factors affecting the micro-emulsion polymerization kinetics and the properties of the particles (Tan and Tam, 2008). Despite the many potential applications of polymer latexes obtained by micro-emulsion polymerization, the commercial use of this process has been limited because typical polymer formulations are dilute and require a large ratio of surfactant to monomer. The surfactant concentrations usually exceed the amount required for polymer stability. Table 3.1 shows the comparison of the most important properties of conventional emulsions, mini-emulsions and micro-emulsions.

3.4.2 Group transfer polymerization (GTP)

Group transfer polymerization is the most suitable polymerization mechanism for methacrylates. Propagation involves reaction of a terminal silyl ketene acetal with a monomer by Michael addition during which the silyl group transfers to the added monomer thus creating a new terminal silyl ketene acetal group. In this kind of polymerization it is common to

use 1-methoxy-1-(trimethylsiloxy)-2-methylpro-1-ene (MTS) as initiator and a carboxylic acid salt as catalyst. The number of growing polymer chains corresponds to the amount of MTS used and chain growth stops when the monomer is depleted. Addition of a new monomer at this point starts chain growth again to produce a block copolymer.

Bütün *et al.* (2008) used GTP technique to obtain branched statistical copolymers by copolymerizing either *N,N*-dimethylaminoethylmethacrylate (DMAEMA) or *N*,*N*-diethylamino ethyl methacrylate (DEAEMA) with ethylene glycol dimethacrylate (EGDMA) in tetrahydrofuran (THF) at 20°C. GTP allows good control over both the primary chain length and the molecular weight distribution as compared with branched vinyl polymers synthesized by conventional radical polymerization. Branched diblock copolymers of DMAEMA and DEAEMA were prepared by sequential monomer addition with EGDMA being used to achieve branches in either the DMAEMA block, the DEAEMA block or in both blocks.

Yamasaki and Patrickios (2003) utilized GTP to synthesize cross-linked homopolymer networks of DMAEMA of various molecular weights. The initiator used for polymerization was MTS while tetrabutylammonium bibenzoate was used as the catalyst. EGDMA was the cross-linker used for the network synthesis at an eight-fold molar excess with respect to the initiator.

3.4.3 Reversible addition-fragmentation chain transfer (RAFT) polymerization

Reversible addition-fragmentation chain transfer (RAFT) radical polymerization allows the synthesis of well-defined macromolecular architectures with a relatively low polydispersity index (Gregory and Stenzel, 2012b; Smith *et al.*, 2010). RAFT polymerization achieves their controlled character due to a reversible chain transfer which reduces the number of radicals and thus reduces the occurrence of termination reactions (Lowe and McCormick, 2007).

A conventional RAFT polymerization contains three primary components – a monomer, a radical initiator and a chain transfer (RAFT) agent. Briefly, the initiator generates radicals, which propagate into polymeric chains. The RAFT agent caps the radicals on the end of the growing chains, temporarily stopping propagation. This capping process is reversible. When the RAFT agent is eliminated from the chains, growth continues. Since the total amount of time spent capping and uncapping is minuscule relative to that spent propagating, all chains essentially grow incrementally at the same rate (Keddie *et al.*, 2012; Yu *et al.*, 2009a).

Reducible PDMAEMAs were synthesized by RAFT polymerization and represent promising carriers of therapeutic nucleic acids (Zhao and Ni, 2005; Zhu *et al*., 2010). Acid-cleavable core-shell-like polymeric colloidal systems for the delivery of hydrophobic drugs at slightly acidic sites were prepared using the acid-labile microgel method and RAFT-mediated seeded dispersion polymerization (Lansalot *et al*., 2002). Bisacrylate acetal cross-linker was copolymerized with *n*-BA in the presence of a RAFT agent, which yielded cross-linked spherical particles with diameters in the range of 150–500 nm. The cleavage of the particles depended on the pH of the medium. In order to cover the hydrophobic surface of the particles, polyethylene glycol acrylate (PEG-A) was grafted to poly(butyl acrylate) (P(BA)) via a RAFT agent on the particle surface. The physico-chemical and functional features support the potential value of the acid-cleavable poly(BA) core–poly(PEG-A) shell particles as carriers for delivery of hydrophobic drugs at acidic sites (Chan *et al*., 2006).

3.4.4 Atom transfer radical polymerization (ATRP)

Atom transfer radical polymerization is one of the most robust controlled/living radical polymerization (CRP) techniques, allowing for the controlled polymerization of various vinyl and acrylic monomers under mild conditions (Matyjaszewski, 2009). An ATRP initiating system consists of an alkyl halide initiator and a transition metal catalyst in the lower oxidation state, copper being the most commonly studied metal (Braunecker and Matyjaszewski, 2007). ATRP is a result of the formation of radicals that can grow, but are reversibly deactivated to form dormant species. Reactivation of the dormant species allows the polymer chains to grow again, only to be deactivated later. The radical formation occurs by action of a transition metal catalyst that activates the organic initiator or dormant species by obstructing the halide at the chain end. Using ATRP, polymers with controlled molar masses and small polydispersities can be obtained (Mespouille *et al*., 2008).

Satturwar *et al*. (2007) prepared block copolymers of PEG and t-butyl methacrylate, iso-BA, *n*-BA or propyl methacrylate by ATRP. They obtained pH-sensitive micelles by hydrolysis of t-butyl groups. The poorly water-soluble drug candersartan cilexetil (CDN) was incorporated in the micelles, and the *in vitro* drug release as a function of pH was studied. The entrapment efficiency of CDN was found to be above 90%. The release of CDN from pH-sensitive micelles increased with increase in pH from 1.2 to 7.2. Thus, this block copolymer synthesized by ATRP can form micelles which exhibit high loading capacities of CDN and release of drug in a pH-dependent response.

Another example of pH-responsive polymer synthesized by ATRP was the PAA and Pluronic P85 block copolymers (P85PAA) prepared by Tian

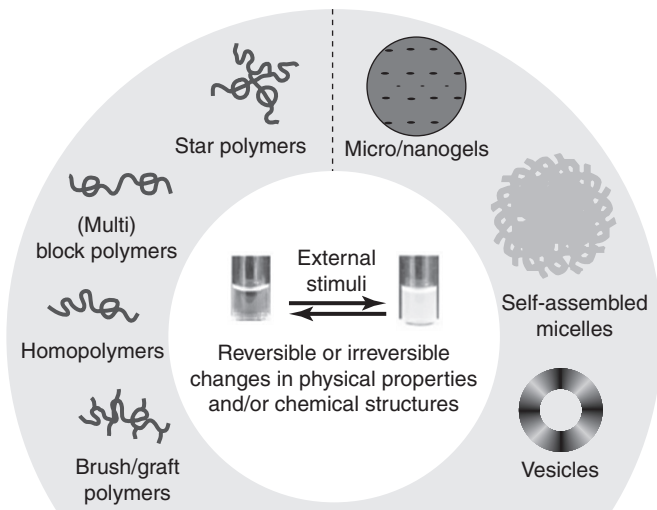
et al. (2007). Physically encapsulated DOX in pluronic micelles enhanced drug delivery to solid tumors and reduced side-effects. The P85PAA-DOX complex formation and drug loading were strongly dependent on the length of the PAA segment and pH. Protonation of carboxyl groups in the PAA segment at pH < 7.3 reduced the binding sites of DOX onto P85PAA chains resulting in a diminished DOX uptake at low pH. Thus, DOX release from the complex is a pH-responsive process where the protonation of carboxyl groups in mildly acidic conditions resulted in a faster dissociation of copolymer-DOX complex, leading to increased release of DOX at pH 5.0 (Bajpai *et al*., 2008).

3.5 Different architectures of pH-responsive polymers

The most common pH-sensitive polymer structures described in the literature are: linear homopolymers or copolymers, amphiphilic block copolymers which form micelles, grafted copolymers, polymer brushes, star and dendritic polymers, NPs, vesicles or HGs (Fig. 3.4). Some of these examples are explained in this section.

3.5.1 pH-responsive amphiphilic block copolymers

Block copolymers are promising candidates for preparing responsive soft materials due to their self-assembling properties. In solution, amphiphilic



3.4 Different architectures of pH-responsive polymers. (*Source*: Reprinted and adapted from Reyes *et al.*, 2013, copyright 2013, with permission from The American Society of Chemistry.)

block copolymers can self-assemble into micellar structures, such as spheres, which can be used as nano-reactors and stimuli-responsive materials. pH-responsive amphiphilic block copolymers contain a number of ionizable groups in their main chains and pendants; therefore their domains can be tuned to respond to aqueous environments. When the pH value is changed, these groups can accept or donate protons in aqueous solution to yield polyelectrolytes, weak polyacid or weak polybase, depending on their structures and the pH values (Xu *et al*., 2006).

pH-sensitive diblock copolymers of polyethylene glycol and t-butylmethacrylate (tBMA), ethyl acrylate (EA) or *n*- BA were synthesized by ATRP (Sant *et al*., 2004). The poorly water-soluble drug progesterone (PRG) was incorporated by dialysis or oil-in-water (O/W) emulsion methods. PRG release was evaluated *in vitro* as a function of pH. The PRG release from supramolecular assemblies increased when the pH of the release medium was raised from 1.2 to 7.2. The results suggest that these supramolecular assemblies with high drug loadings and pH-dependent release kinetics can potentially enhance the oral bioavailability of hydrophobic drugs.

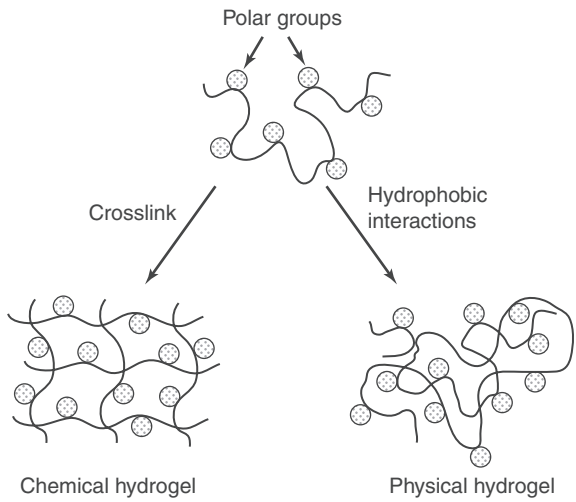
Various groups have reported the microencapsulation of drugs in polymeric micelles, which have a core-shell architecture that self-assemble in aqueous media due to their amphiphilic block copolymers. PEG is a popular choice for a shell due to its biocompatibility and water solubility. Oishi and co-workers (Oishi and Nagasaki, 2007) described a PEGylated nanogel for DOX delivery. The nanogel was constructed from a cross-linked, pH-sensitive polyamine core and tethered PEG chains. Under high or neutral pH the PEG gel showed a collapsed structure; however, as the pH decreased it demonstrated an increased volume transition and subsequent release of drugs. Shim *et al*. (2006) prepared a novel pH-sensitive and thermo-sensitive block copolymer by adding the pH-sensitive sulfamethazine oligomer (SMO) to the thermo-sensitive block copolymer of poly(ε-caprolactone-co-lactide)-PEG-poly(ε-caprolactone-co-lactide) (PCLA–PEG–PCLA). The sulfonamide-modified block copolymer exerted a distinctive sol–gel transition in response to small changes in pH at body temperature. At pH 8.0, the sulfonamide-modified block copolymer solution maintained its sol phase for about 2 h at body temperature, but rapidly formed a gel in physiological conditions (pH 7.4 and 37°C within 5 min). Armes and co-workers (Du *et al*., 2011) prepared biocompatible pH-responsive micelles and vesicles with pH-tunable permeability. Reyes-Ortega *et al*. (2013a) prepared low molecular weight heparin (LMWH) NPs by W/O emulsion and inversion phase emulsion (O/W) using well-defined PMMA-*b*-PMAETMA block copolymers synthesized by RAFT polymerization. PMMA-*b*-PMAETMA sequences resulted in a self-assembled core-shell NP in water with a positively charged surface which interacts with negatively sulfated and carboxylate groups of LMWH. Stupp and co-workers (Shah *et al*., 2010) went ahead preparing

supramolecular structures using peptide amphiphiles (PAs), bioactive materials that self-assemble from aqueous media into supramolecular nanofibers of high aspect ratio. These molecules, targeted to serve as the components of artificial extracellular matrices, consisted of a peptide segment covalently bonded to a more hydrophobic segment such as an alkyl tail. They carried out an *in vitro* study showing that self-assembling PA scaffolds can support human mesenchymal stems cell (hMSC) viability and chondrogenic differentiation and lead to upregulated gene expression of cartilage. They demonstrated *in vivo* that PA synthesized with a peptide-binding sequence to TGFβ-1 significantly enhanced the regenerative potential of microfracture-treated chondral defects. Kataoka and his group synthesized charge-conversion micelles for the delivery of proteins (Kataoka *et al*., 2012). The attachment of methyl maleate groups to the aspartate block of a PEG-pAsp amphiphile resulted in micelles which were negatively charged under physiological conditions. When these micelles entered into the cell they cleaved off the methyl maleate group, resulting in a free, positively charged amine with the release of the encapsulated protein, due to the response to the endosomal pH (Miyata *et al*., 2007).

3.5.2 pH-responsive hydrogels and microgels

Hydrogels are polymeric tridimensional networks that do not dissolve in water at physiological temperature and pH. Polymer microgels are cross-linked particles which form a network structure like HGs. Both systems may absorb from 10–20% up to thousands of times their dry weight in water. They are called reversible or physical gels when the networks are held together by molecular entanglements and/or secondary forces including ionic, H-bonding or hydrophobic forces. In the opposite case, they are called permanent or chemical gels when they are covalently cross-linked networks (Hoffman, 2012) (Fig. 3.5). A mixture of physical and chemical cross-linked networks forms a SIPN. Gels exhibiting a phase transition in response to change in external conditions such as pH, ionic strength, temperature and electric currents are known as 'stimuli-responsive' or 'smart' gels.

The network porosity of these gels changes with electrostatic repulsion. Ionic HGs containing carboxylic or sulfonic acid groups show either sudden or gradual changes in their dynamic or equilibrium swelling behavior as a result of changing the external pH. The degree of ionization of these gels depends on the number of pendant acidic groups in the gel, resulting in increased electrostatic repulsions between negatively charged carboxyl groups on different chains. This, in turn, results in increased hydrophilicity of the network and greater swelling ratio at high pH. Conversely, HGs containing basic pendant groups, such as amines, ionize and show electrostatic repulsion at low pH (Zhao *et al*., 2009). Calcium alginate is an example



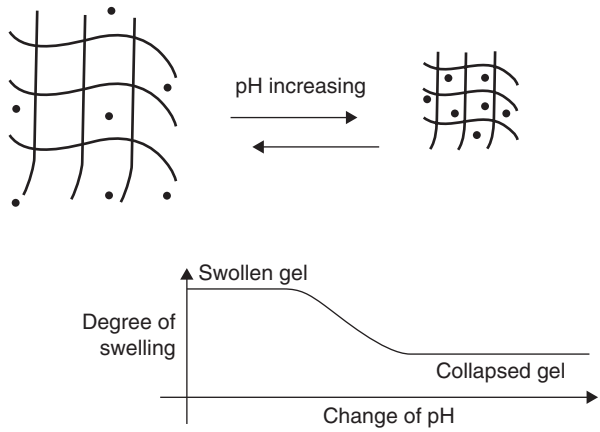
3.5 Scheme of a chemical (permanent) and a physical (reversible) hydrogel structures.

of this type of hydrogel. Lim and Sun prepared calcium alginate capsules coated with a complex coacervate of alginate–PLL to stabilize the capsule. These polyion complex HGs have become attractive as tissue engineering matrices (Teramura and Iwata, 2010). Na *et al*. (2006) described pH-sensitive polymers containing sulfonamide groups, which show changes in swellability and solubility depending on pH (Park *et al*., 2013). These pH-dependent HGs may be grouped into two main classes: (a) cationic HGs and (b) anionic HGs.

Cationic hydrogels

Cationic HGs swell and release a drug in the low pH environment of the stomach (Fig. 3.6). Risbud *et al*. (2000) prepared a pH-sensitive, chitosan/poly(vinyl pyrrolidone) (CS/PVP)-based, controlled drug release HG using air-dried and freeze-dried amoxicillin. Porous freeze-dried hydrogel exhibited superior pH-dependent swelling properties over non-porous air-dried HGs. Freeze-dried membranes released around 73% of the amoxicillin (33% by air-dried) in 3 h at pH 1.0, and thus had the better drug release properties.

Gupta and Ravi Kumar (2000) described spherical cross-linked beads using chitosan, glycine and glutaraldehyde. The swelling behavior of the beads was studied as a function of time in solutions of different pH. The release experiments were performed using thiamine hydrochloride as the model drug. The chitosan beads showed a pH-dependent swelling behavior which makes them appropriate for delivery of drugs in an acidic environment.



3.6 Cationic hydrogel swelling behavior: at low pH the drug is released due to the swelling of the polymer network. (*Source*: Reprinted and adapted from Reyes *et al.*, 2013, copyright 2013, with permission from Elsevier B.V.)

Anionic hydrogels

Anionic HGs have pendant groups such as carboxylic groups and can be used to develop formulations that release drugs in a neutral or basic pH environment. HGs of polyanions (e.g., PAA) cross-linked with azoaromatic cross-linkers were developed for colon-specific drug delivery (Ghandehari *et al.*, 1997). The swelling increases as the hydrogel passes down the intestinal tract due to an increase in pH leading to ionization of carboxylic groups.

The kinetics of the swelling of HGs can be controlled by changing the polymer composition and varying the ionic concentrations, which can be changed as the pH of the environment changes. Some pendant groups, such as *N*-alkanoyl (e.g., propionyl, hexanoyl and lauroyl) and *o*-acylhydroxylamine moieties, can be hydrolyzed as the pH changes from acidic to neutral; and the rate of side-chain hydrolysis is dependent on the length of the alkyl moiety. Kim and Peppas (2003b) studied the mesh sizes of the HGs at different pH values and they observed MAA HGs with a very small mesh at pH 2.2 (18–35 Å) in the collapsed state which became very large meshes (70–111 Å) in the swollen state at pH 7.0. In addition, as the MAA content of the feed monomers was increased, the mesh size decreased at pH 2.2 but increased at pH 7.0. When the cross-linking ratio of the copolymer increased, the swelling ratio decreased at both pH 2.2 and pH 7.0.

In another study it was found that an increase in the degree of ionization contributed to the electrostatic repulsion between adjacent ionized groups, leading to chain expansion, which affected macromolecular chain relaxation. However, for both P(MAA-co-MAA) and P(MAA-g-Eg) HGs, the

swelling mechanism exhibited little dependence on the copolymer composition of each hydrogel at the same pH (Kim and Peppas, 2003a).

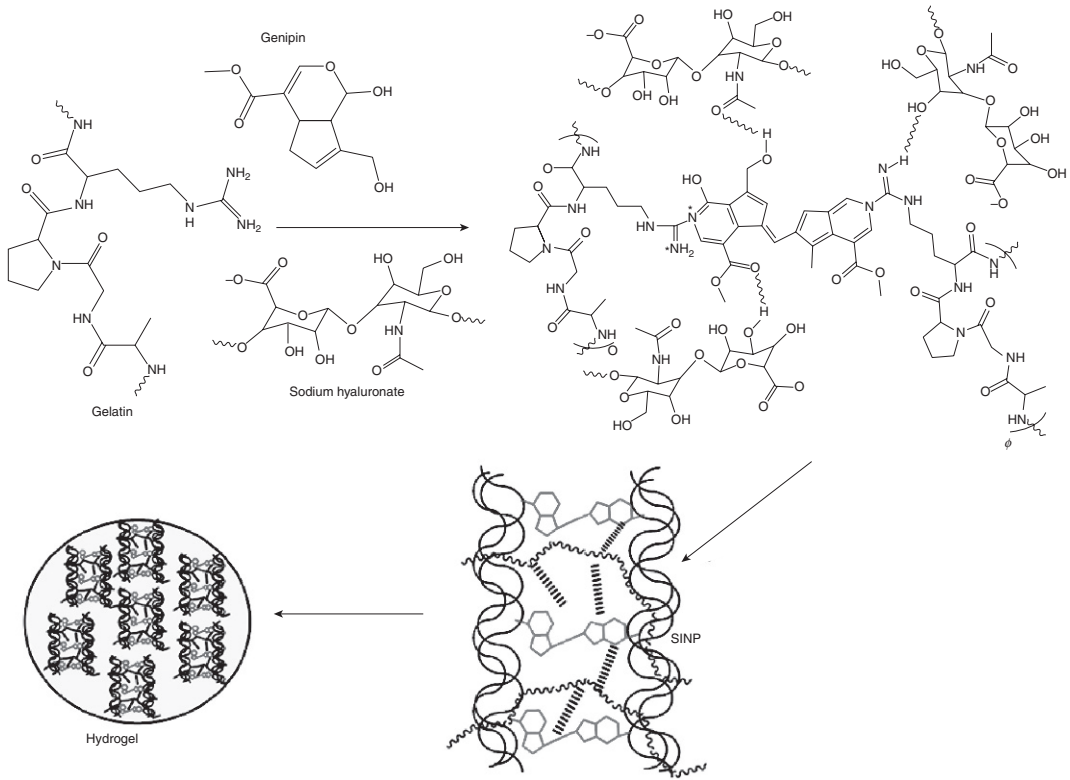
Reyes-Ortega *et al.* (2013b) described a HG formed by gelatin and sodium hyaluronate loaded with LMWH NP. Gelatin was chemically cross-linked with genipin, and sodium hyaluronate entanglements were distributed along the tridimensional network creating a SIPN (Fig. 3.7). The swelling degree of these HGs depends on the pH of the medium, allowing the highest swelling and LMWH release at physiological pH (pH 7.4). These HGs are useful for the development of drug delivery carriers.

3.5.3 pH-responsive polymer brushes

Stimulus-responsive polymer brushes (SRPB) are a category of polymer brushes that exhibit a change in their conformation, surface energy or change state, triggered by an external stimulus such as a change in solvent, temperature, pH, ionic strength, light or mechanical stress. The control and reversible polymer chain conformation and surface energy discovery in SRPB has offered exciting and novel possibilities for the fabrication of adaptive or responsive surfaces and interfaces.

pH-responsive polymer brushes contain ionizable pendant groups that can accept or donate protons in response to an environmental change in pH. These brushes often contain weakly acidic or basic groups with pK_a values around which the degree of ionization is dramatically altered. A rapid change in the net charge of pendant groups causes a dramatic change in the hydrodynamic volume of the polymer chains which ensues from changes in the osmotic pressure exerted by mobile counterions neutralizing the polymer charges. Moreover, pH-responsive polymer brushes are typically also responsive to changes in ionic strength, where screening of repulsive electrostatic interactions increase with increasing ionic strength resulting in brush collapse (Chen *et al.*, 2010).

Zhou and Huck (2005) prepared a pH sensitivity polymer of poly(methacryloyl ethylene phosphate) (PMEP) brushes bearing o-phosphonic acid with two ionization states for switching surface wettability (pK_{a1} in the range of pH 1–2 and pK_{a2} in the range of pH 6–7). The phosphate groups are completely protonated (diacid form) at pH 1, partly protonated when pH is close to 7, and are completely un-protonated (dibasic form) when pH >7. The charges on the brush, the concentration of free counterions, and the degree of swelling can therefore all be tuned via the adjustment of the pH. Another example is poly(2-(methacryloyloxy)-ethyl-trimethylammonium chloride) (PMAETMA) brushes that contain Cl^- counterions. Changing the Cl^- anions with SCN^-, PO_4^{3-} and Cl_4^- anions produces a drastic change in the wetting properties of the substrate (Azzaroni *et al.*, 2007).



3.7 Scheme of a semi-interpenetrating network formed by sodium hyaluronate and gelatin cross-linked with genipin. (*Source*: Reprinted and adapted from Reyes *et al.*, 2013, copyright 2013, with permission from Sociedad Ibérica de Biomecánica y Biomateriales.)

3.5.4 pH-responsive nano- or microparticles

Polymers containing ionizable groups, such as amines and carboxylic acids, are the best candidates for fabricating pH-sensitive nanocarriers. Particulate carriers offer some unique advantages as delivery, sensing and image enhancement agents (Felber *et al*., 2012). These nano/micro-carriers are generally considered for use in the target-specific drug or gene delivery systems to various sites in the body, to improve therapeutic efficacy, while minimizing undesirable side-effects. One advantage of these pH-responsive carriers is their capacity to respond specifically to a certain pathological trigger. Some particles are based on a core and a shell, with different compositions and properties in each layer.

Yao and collaborators (Yao *et al*., 1996) prepared pH-responsive microspheres based on chitosan. In their study, chitosan/gelatin hybrid polymer network microspheres were synthesized by an inverse emulsion method, with glutaraldehyde as the cross-linker. The results showed that drug release occurred only in acidic media and that microspheres have potential as carriers for intelligent drug delivery systems. Chuang *et al*. (2009) prepared NPs of AA and N-Isopropyl acrylamide monomer (NIPAAm) loaded with doxycycline hyclate using surfactant-free emulsion polymerization. They studied the structure, particle size, morphology, surface charge, responsive properties and *in vitro* drug release behavior of the NPs. The release profile of doxycycline hyclate from the drug-loaded NPs at two pH values (7.0 and 2.0) was different, showing faster release at the higher pH.

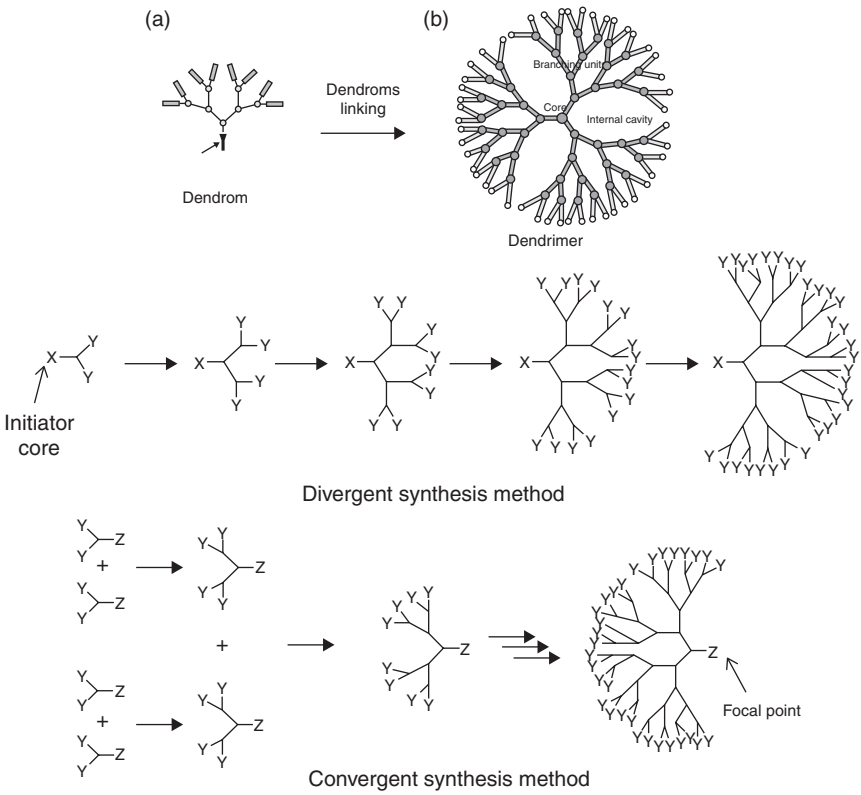
Cui *et al*. (2009) prepared carboxylated chitosan-grafted PMMA NPs for use in insulin delivery. Yu *et al*. (2009b) developed drug-loaded microparticles based on chitosan, alginates and pectin. An alginate with carboxyl groups shrank at low pH but dissolved at high pH. Therefore, the solubility of chitosan was reduced by the alginate network, under low pH conditions. The dissolution of alginates was reduced by chitosan, at high pH. Results showed that drug release at pH 1.2 and 5.0 was slow. The release at pH 7.4 was much faster. These microparticles had a high pH sensitivity and could be potentially used for site-specific protein drug delivery, through oral administration.

Kaminski *et al*. (2008) prepared chitosan microspheres for heparin removal. Heparin is a highly sulfated GAG polysaccharide with excellent anticoagulant activity. This drug is frequently used, but presents several side-effects; therefore after heparin has exerted its activity, it is often necessary to remove it from the blood. Microspheres were synthesized by cross-linking chitosan with genipin by inverse emulsion polymerization. For pH values below 6.5, the microspheres swelled considerably and at pH above 6.5 they shrank by a small extent. Chitosan microspheres bind heparin in water. The kinetics of heparin binding was found to be pH dependent and was faster

and more efficient at a lower pH. Rate and efficiency of heparin adsorption at pH 7.4, which is typical of blood, could be increased by quaternization of chitosan microspheres using glycidyltrimethylammonium chloride. This polymeric material obtained can be potentially useful for heparin removal in biomedical applications.

3.5.5 pH-responsive dendritic polymers

Dendritic architectures (Fig. 3.8a) show very beneficial properties for the development of drug delivery systems and thus many different systems based on dendrimers, dendroms or hyperbranched polymers have been developed (Oliveira *et al*., 2010). In general, one can distinguish between two different release mechanisms from dendritic molecules depending on the way the



3.8 Scheme of (a) dendrom structure and dendrimer architecture and (b) different dendrimer methods of synthesis.

guest was incorporated (Wong *et al.*, 2012) (Fig. 3.8b). Either the guest molecule is complexed in a non-covalent system or it is covalently bound via a pH-degradable linker to the dendrimer. In the case of non-covalent encapsulation the guest is released due to the protonation of internal functional groups or due to the cleavage of the shell. Some dendritic molecules already show pH-responsive behavior without requiring further modification (York *et al.*, 2008). The first dendrimer used for the encapsulation of a guest molecule was a poly(propylene imine) (PPI) dendrimer. Meijer and co-workers modified the PPI dendrimer surface with protected amino acids and encapsulated different guests in the so-called 'dendritic box' (Malik *et al.*, 2000). Poly(amido amine) (PAMAM) dendrimers also show a certain pH-responsiveness due to the protonation of the internal tertiary amines and the primary amines on the surface of the dendrimer (Esfand and Tomalia, 2001). Interestingly, the conformational change in aqueous media does not go along with swelling phenomena which was shown by molecular dynamic simulations: at low pH PAMAM forms a very dense shell, while at high pH it has a rather dense core.

Furthermore, these types of architectures benefit from the enhanced permeation and retention (EPR) effect (Barrett *et al.*, 2009). The direct conjugation of drugs to the dendritic backbone via pH-degradable linkers is also a powerful tool in designing responsive drug delivery systems (Lee and Nan, 2012). Recently a whole library of dendrimer systems was prepared, which contains some pH-responsive dendritic architectures, for example a dendrimersome formed via diacetal connections resulting in a potential candidate as a pH-responsive drug delivery device (Peterca *et al.*, 2011).

3.6 Applications

The human body presents variations on pH along the gastrointestinal (GI) tract, and also in some specific areas like certain tissues (and tumoral areas) or sub-cellular compartments. The polymer–polymer and the polymer–solvent interactions (the solvent in biomedical applications will usually be water) show an abrupt re-adjustment in small ranges of pH, and this is translated to a chain transition between extended and compacted coil states.

Changes in polymer conformation are manifested by alterations to surface wettability, charge state or solubility. These unique properties of pH-responsive polymer systems consequently make them very useful in biorelated applications such as drug delivery, biotechnology, chromatography, and they have been used in several biomedical applications, such as drug and gene delivery systems and glucose sensors. We report in this section the most attractive examples reported in the last years.

3.6.1 Drug delivery systems (DDS)

pH-sensitive polymers are a very promising platform for drug delivery as the body exhibits extreme physiological pH variations throughout the GI tract, which can range from pH 2 in the stomach, pH 7 within the colon and up to pH 8.2 in the lower duodenum. Therefore, based on the changes of pH it is possible to create pH-sensitive micro-carriers that are stable at low pH values in the stomach and release their content only under neutral or alkaline conditions. This condition makes pH-sensitive polymers ideal for colon-specific drug delivery. The most common approach utilizes enteric polymers that resist degradation in the acidic environment and release their drug in alkaline media due to the formation of salt. There are several examples of these kind of polymers already commercialized, including Eudragit L®, Eudragit S® from Evonik Röhm GmbH (based on methacrylic acic and methyl methacrylate) or CMEC from Freund Sangyo Co., Ltd; CAP from Wako Pure Chemicals Ltd.; HP-50 and ASM from Shin-Etsu Chemical Co., Ltd. (derived from cellulose). A large number of polysaccharides such as amylose, guar gum, pectin, chitosan, inulin, cyclodextrin, chondroitin sulfate, dextran and locust beam gum, have also been investigated for colon-specific drug release.

Other pathological states are associated with changes in pH environment; ischemia, infection, inflammation and cancer are often linked to acidosis. For example, invasive cancers proliferate rapidly and require a constant nutritional supply. However, due to an often disorganized blood supply there is a lack of nutrient and oxygen transfer resulting in a hypoxic environment and production of lactic acid. This local acidosis therefore represents an ideal target for chemotherapeutic drugs within a pH-sensitive carrier.

Researchers have designed more sophisticated pH-sensitive polymers in order to take advantage of the pH changes that occur in nature. These materials are inspired by living organisms trying to mimic their response mechanisms. Sauer *et al*. (2001) have reported the synthesis of pH-sensitive hollow nanocontainers inspired by virus particles. The PAA vehicles were synthesized by vesicular polymerization and emulsion polymerization (using core-shell latex particles). These nanocapsules combine the protective ability of the nanocontainers in combination with controlled permeability and therefore can be used to trigger the release of encapsulated materials from the inner core.

Bellomo and co-workers prepared a new type of synthetic vesicle with a high degree of architectural control made of amphiphilic block copolypeptides (Bellomo *et al*., 2004). The hydrophilic block was made of lysine, augmented with a few water-soluble ethylene glycol units, and the hydrophobic block was constituted by leucine peptide. The synthetic polymer forms a supramolecular structure highly sensitive to environmental signals

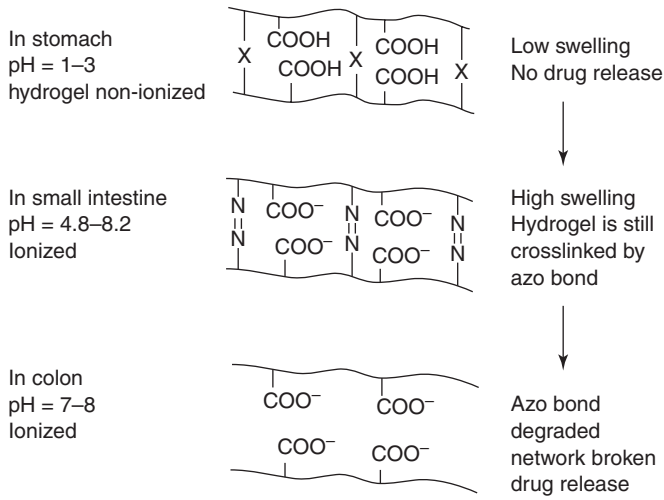
and able to respond precisely to pH changes and actuate as a drug delivery carrier.

Most of the drug release intelligent systems are driven by the phase–volume transition. However, Liu and co-workers reported the preparation of novel β-cyclodextrin microgels for drug delivery applications driven by inclusion effects (Huang *et al.*, 2012). pH-sensitive polymers have also been incorporated into organic–inorganic composites obtaining materials that presented both the advantages of inorganic materials (high mechanical stability) and conventional polyelectrolyte capsules (the controlled release/uptake properties of the capsule shell resulting from changes in, among others, pH values and ionic strength). Shchukin *et al.* (2003) presented the synthesis and characterization of new inorganic/organic composites capsules where the inorganic particles acted as building blocks glued together by a pH-sensitive polyelectrolyte. Besides the common applications of polyelectrolyte capsules in controlled release, these composite inorganic/organic capsules can also be applied as mechanically stable microreactors for enzymatic reactions and synthesis employing gas phase reagents, in the form of hollow catalytically microcontainers. The inorganic part can also find biomedical application which, together with encapsulated drug material, can provide synergistic curing effects, such as the application of hydroxyapatite-containing capsules in bone repair. These capsules can also be used as protective solid microcontainers due to their ability to preserve the initial spherical shape and controlled release properties upon drying (Shchukina and Shchukin, 2012).

In particular, the pH-responsive block copolymers are very important because they can form NPs, for example, polymeric micelles, vesicles or hollow nanospheres, in aqueous media via the changing environment, and further provide a variety of applications for the biomedical fields.

pH-sensitive HGs have been applied most frequently in drug delivery systems. For polycationic HGs, the swelling is minimal at neutral pH, thus minimizing drug release from the HGs. This property has been used to prevent release of foul-tasting drugs into the neutral pH environment of the mouth. For example, when caffeine was loaded into HGs made of copolymers of methyl methacrylate and DMAEMA, it was not released at neutral pH, but released at zero-order at pH 3–5 where DMAEMA became ionized (Siegel *et al.*, 1988). Polycationic HGs in the form of a SIPN have also been used for drug delivery in the stomach. SIPNs of cross-linked chitosan and PEG showed more swelling under acidic conditions (as in the stomach) (Satish *et al.*, 2007). This type of HG would be ideal for localized delivery of antibiotics, such as amoxicillin and metronidazole, in the stomach for the treatment of *Helicobacter pylori* (Gupta *et al.*, 2002).

HGs made of PAA or PMA can be used to develop formulations that release drugs in a neutral pH environment (Qiu and Park, 2012). The extent of swelling increases as the HGs passes down the intestinal tract



3.9 Schematic illustration of oral colon-specific drug delivery using biodegradable and pH-sensitive hydrogels. (*Source*: Reprinted and adapted from Reyes *et al.*, 2013, copyright 2013, with permission from Elsevier B.V.)

due to increased pH leading to ionization of the carboxylic groups. Only in the colon, however, can the azoaromatic cross-links of the HGs be degraded by azoreductase produced by the microbial flora of the colon, as shown in Fig. 3.9. The polymer composition can be changed as the pH of the environment changes. Some pendant groups, such as *N*-alkanoyl (e.g., propionyl, hexanoyl and lauroyl) and O-acylhydroxylamine moieties, can be hydrolyzed as the pH changes from acidic to neutral values and the rate of side-chain hydrolysis is sensitive on the length of the alkyl moiety.

3.6.2 Gene carriers

One of the most promising applications of pH-sensitive polymers is as non-viral gene carriers. Naked DNA is very difficult to incorporate into the cells because it is negatively charged and it is very large at physiological conditions. Liposomes and polycations are the two major classes of chemical (non-viral) gene delivery methods to condense DNA in charge-balanced NPs that can be carried into cell compartments (Park *et al.*, 2012). Condensation of negatively charged plasmid DNA with cationic polymers or lipids reduces the size of plasmids several thousand base pairs in length to NPs of 100–200 nm diameter, protects the plasmid DNA against extracellular degradation and increases the cellular uptake of plasmid DNA via charge interactions (Green *et al.*, 2008). Godbey and Mikos (2001) reviewed

some of the advances in non-viral gene delivery research describing the use of PEI and PLL as two of the most successful candidates for this application. PEI is a highly polycationic synthetic polymer that condenses DNA in solution, forming complexes that are readily endocytosed by many cell types. Chitosan, a biocompatible and resorbable cationic aminopolysaccharide, has also extensively been used as a DNA carrier. Lim and co-workers (Lee *et al.*, 2011), prepared induced pluripotent stem (iPS) cells from fibroblasts using a non-viral MNP-based transfection method that employs a biodegradable cationic polymer PEI-coated superparamagnetic NP. These magnet-based nanotransfection provided a safe method for use in the generation of virus-free and exogenous DNA-free iPS cells, which is crucial for future clinical applications in the field of regenerative medicine. Kataoka (2004) developed polymeric micelles as nanocarriers for gene and drug delivery based on DOX-conjugated block copolymer poly(ethylene glycol)-poly(aspartame hydrazinedoxorubicin) (PEG-p(Asp-Hid-dox). The polymer retained drugs and genes at physiological pH and released the drugs as pH decreased below 6.0.

Anionic polyelectrolytes have been used in the development of new intracellular delivery systems by membrane destabilizing mechanisms (Wasungu and Hoekstra, 2006). These polymers can be tailored to interact actively with phospholipid membranes upon external stimulation, such as acidification of the surrounding medium. This strategy has been exploited to improve the cytoplasmic delivery of biomolecules (DNA, proteins) that enter cells by endocytosis and end up in acidic organelles (Gupta *et al.*, 2005).

Hoffman's group has dedicated great efforts to obtain new delivery systems to efficiently introduce biomolecules to intracellular targets (Henry *et al.*, 2006; Pack *et al.*, 2005). They mimicked the molecular machine of some viruses and pathogens that are able to sense the lowered pH gradient of the endosomal compartment and become activated to destabilize the endosomal membrane. This mechanism enhances protein or DNA transport to the cytoplasm from intracellular compartments such as endosome.

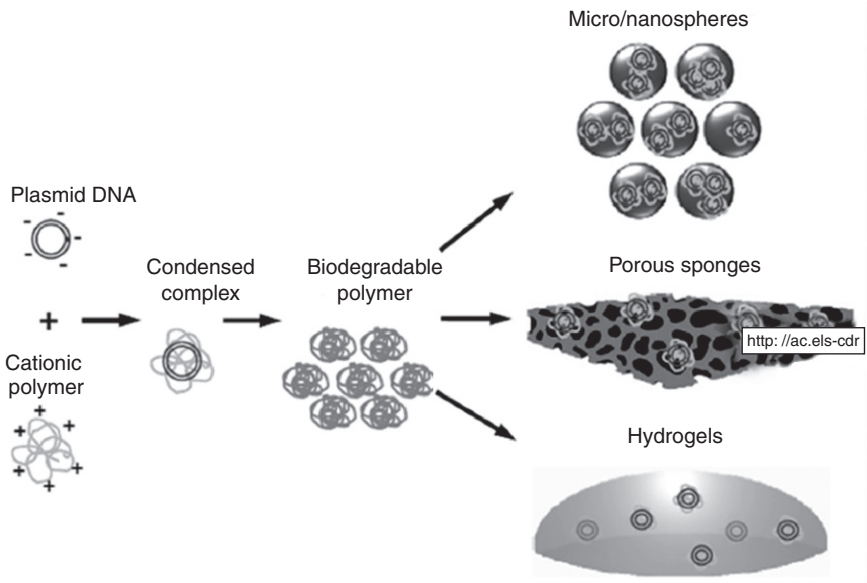
Recently, intracellular delivery of genes has gained extensive attention due to recent progress in biotechnology and its medical potentials. The strategy is to give cells the capability of producing therapeutic proteins directly from the delivered genes. Park *et al.* (2012) reviewed several strategies for the treatment of ischemic diseases through clinical trials employing non-viral gene therapy. The genes encoding proteins that can stimulate angiogenic signal transduction have been administered by cationic polymers, lipids, liposomes, three-dimensional scaffolds, or even without any delivery vehicles.

Various types of cationic polymers have been tested for gene transfection into vascular or cardiac cells, including linear or branched PEI, polyamidoamine dendrimer, PLL, and poly(β-amino esters). The positively charged polymer-DNA complexes enter the cytoplasm by binding

to the negatively charged cell membrane and endocytosis. Due to their buffering capacity between pH 5.0 and 7.2, the endosome is ruptured by the increased osmotic pressure after acidification of the endosome, and the internal contents are released. Therefore, cationic polymers can efficiently deliver DNA into the cytoplasm without the help of any colipids as a liposomal compartment. Modification of cationic polymers with additional molecules, such as heparin (Jeon *et al*., 2008) and cholesterol (Katz *et al*., 2011), may further improve their delivery efficiency and reduce their toxicity. Pseudo-peptides, poly(L-lysine iso-phthalamide) and poly(L-lysine dodecanamide) containing carboxylic side groups, were utilized as effective DNA carriers. These polymers changed their conformations into hypercoiled structures at endosomal pH, leading to high transfection efficiency (Ho *et al*., 2011). A comb-type pH-responsive polybase DNA carrier was reported, consisting of a poly(N,N-diethylamino ethyl methacrylate) (PDEAEMA) backbone and PLL side chains (Xu *et al*., 2009). PLL has usually been employed as a cationic polymer capable of forming complexes with DNA and protecting DNA via ionic interactions. In this study, they combined a pH-responsive, membrane-disruptive property into PLL by introducing PDEAEMA. However, PDEAEMA has its transition pH value at around physiological pH. The transition pH value of PDEAEMA should be adjusted to the endosomal environment via incorporating a hydrophobic group or replacing it with an appropriate pH-responsive polymer.

Complexation of pH-responsive polymers with liposomes is another strategy to enhance transfection efficiency (Coelho *et al*., 2010). A pH-responsive polymer bearing long hydrophobic pendant groups has been designed to be anchored onto liposomes, improving the ability of liposome complexes to disrupt the lipid bilayer with its serum stability (Sakaguchi *et al*., 2008). For example, the pH-responsive polymer (a copolymer of N-isopropylacrylamide (NIPAA), *N*-glycidylacrylamide and *N*-octadecylacrylamide) was combined with a large unilamellar niosome (non-ionic surfactant vesicle), bilayer consisting of polyoxyethylene-3-stearyl ether (POE-SE) surfactant linked with cholesterol, instead of liposome (Patent EP 1069910 A1).

HGs could also be a reservoir of therapeutic genes for local sustained delivery (Fig. 3.10). For example, gelatin HGs have been shown to potentiate the angiogenic effects of FGF-4 plasmids by prolonging DNA release. The injection of gelatin HGs loaded with FGF-4 plasmid-promoted blood vessel formation and blood flow in ischemic limb muscle is more effective than naked FGF-4 plasmids (Kasahara *et al*., 2003). Another example was alginate HGs which were used for the encapsulation and delivery of a PEI–vascular endothelial growth factor (VEGF) plasmid complex. Alginate hydrogel was engineered to degrade via physical dissociation between cross-linking segments of polymers and hydrolytic polymer



3.10 Scaffold-mediated gene delivery. Plasmid DNA condensed with cationic polymers can be encapsulated into various types of 3D polymeric scaffold systems (micro/nanospheres, porous sponges, or hydrogels) for sustained gene delivery. (*Source*: Reprinted and adapted from Reyes *et al.*, 2013, copyright 2013, with permission from Elsevier B.V.)

chain breakage. Hydrogel-mediated gene transfer of hypoxia-inducible factor-1α was evaluated for its local induction of angiogenesis (Kong *et al.*, 2008).

Particle formulations can be also used for sustained gene delivery (Fig. 3.10). In several studies, plasmid DNA was loaded into biodegradable particles by using a double emulsion technique (Akiyama *et al.*, 2010; Kang *et al.*, 2008).

3.6.3 Biosensors

One of the most popular applications of pH-sensitive polymers is the fabrication of insulin delivery systems for the treatment of diabetic patients (Hu and Liu, 2010). Insulin has to be delivered in an exact amount at the exact time of need, which makes it more difficult than delivering other drugs. When there is a rich glucose environment, such as the bloodstream after a meal, the oxidation of glucose to gluconic acid catalyzed by glucose oxidase (GluOx) can lower the pH to approximately 5.8. This enzyme is the most widely used in glucose sensing, and makes possible the use of different types of pH-sensitive HGs for modulated insulin delivery.

The glucose responsive polymer system can provide self-regulating insulin in response to the concentration of glucose in the blood, which can control the concentration of insulin within a normal range. One strategy for this purpose is based on pH-responsive polymer HGs, with entrapped GluOx, catalase and insulin (Priya *et al*., 2009). A polyacid such as gluconic acid was demonstrated to be a useful pH-responsive polymer-based glucose release system (Kang and Bae, 2002). In this system, the oxidized gluconic acid lowers pH and protonates acidic groups of polyacids, leading to the shrinkage of the HGs and the release of the entrapped insulin through porous molecular valves for microfluidics. A glucose-sensitive hydrogel containing derivatives of p-aminobenzene sulfonamide was synthesized by copolymerization with polymerizable sulfadimethoxine monomer, *N,N*-dimethylacrylamide (DMAAM) and sucrose particles that were used as a porogen (Kang and Bae, 2003).

Ruan *et al*. (2003) presented a mass-changing pH-responsive HG that increased sensitivity by increasing the fraction of the AA in the poly(acrylic acid-co-isooctyl acrylate) copolymer. Maruyama *et al*. (2008) described a hydrogel film made of UV photosensitive resin. The output is a change in color of the HGs that is one of the most easily recognizable changes for humans to detect (Deligkaris *et al*., 2010).

Glycopolymers contain concentrated saccharide moieties on the polymer chain, which exhibit strong interactions with lectins, a plant protein bearing a high affinity for specific sugar residues (Mahkam, 2007). Poly(ethylene oxide)-block-poly(2-glucosyl-oxyethyl acrylate) (PEO-b-PGEA) was designed for a glucose responsive micellar structure, which can disrupt the micellar structure and release entrapped insulin when the glucose concentration in blood is high. This diblock copolymer was synthesized by ATRP with a methoxy-end-capped poly(ethylene oxide) macroinitiator (Chiappetta and Sosnik, 2007).

pH/temperature-sensitive polymers P(NIPAA-co-BMA-co-AA) were used to prepare insulin releasing beads via loading in aqueous solution (Torres-Lugo and Peppas, 2000). At acidic pH and body temperature, the beads were insoluble, and thus no drug was released in the stomach. At pH 7.4 and body temperature, the low molecular weight hydrophilic polymeric beads displayed a hump-like profile and dissolved within 2 h (bead dissolution-controlled release mechanism), while the high-molecular weight hydrophilic polymeric beads swelled and released insulin slowly over a period of 8 h.

The applicability of a pH-sensitive hydrogel for sensors can be extended greatly by adding an intermediate step where an analyte is converted to pH, as for example, in the hydrogel-based P_{CO2} sensor where CO_2 gas forms carbonic acid in water, resulting in a change in the pH and thus indirectly in the volume of the pH-sensitive HGs (Herber *et al*., 2005).

3.7 Conclusion

This chapter has attempted the compilation of the most recent advances performed in the field of pH-sensitive polymers and their application as drug, gene delivery carriers and biosensors. pH-sensitive HGs are basically polyelectrolytes of either charge and operate by widening of the mesh sizes of their network resulting from repulsive forces developed due to ionization or protonation of the constituent polymer chains. Whereas cationic polyelectrolytes like chitosan work well in the low pH environment of the stomach, anionic HGs such as PAA, PMA, etc, work efficiently in the alkaline environment of the colon. Depending on the end-application and desired functioning, various geometrical and chemical architectures like polymer brushes, amphiphilic block copolymers, HGs and NPs have been designed to achieve high performance. Polymer brushes find application in stimuli-responsive surfaces, chemical gates, cell-growth confinement, etc. Block copolymers and, in particular, amphiphilic block copolymers, zwitterionic polymers, di- and triblock copolymers, biodegradable aliphatic polyesters, polysilane-b-PMAA, etc., have been prepared by specific well-controlled methodologies such as GTP, ATRP and RAFT polymerization, and they have been evaluated for many biomedical applications. Polymer HGs of different chemical architecture with novel physico-chemical properties have shown potential to find applications as promising drug-carrying vehicles in various drug delivery technologies. Although their synthesis and *in vitro* study seems to be simple, from the view point of *in vivo* applications the polymer systems need to be judged with extreme care before they can be accepted ultimately for commercial applications.

The use of pH-sensitive polymers in drug delivery technologies has not only to focus on the possible medical benefits but must also consider the economic aspects of the developed materials and/or technology. Huge efforts in synthetic polymer chemistry must be undertaken to design tailor-made macromolecular systems that will offer novelty in their operation and performance. Above all, the systems developed must be acceptable to the patient community who are the end-users of any successful research and technology. Since pH-responsive materials are disposed to typical experimental conditions, there is large scope for synthetic polymer chemistry to design multi-responsive delivery systems. Despite the tremendous research applied to achieve high performance technologies, a number of aspects still remain to be worked on:

- Designing of drug delivery systems with multistimuli-responsive potential.
- More precise synthetic routes to get responsive materials with greater responsive sensitivity.

- Assurance of economic viability so that these devices could be produced on a large commercial and population scale.
- Design of more localized drug delivery systems.
- Oral delivery of insulin using body-friendly natural polymers with enhanced absorption in blood.

In the future it will be necessary for further interdisciplinary work combining the approaches of chemical engineering, biology and material science to develop stimuli-responsive materials and their range of applications, particularly in terms of cellular therapies and regenerative medicine. pH-responsive polymers may well be suited to applications in drug delivery for clinical therapies and the manipulation of cells including neuronal and stem cells.

3.8 Future trends

The next generation of biomaterials looks toward the development and clinical use of smart materials which will allow better control over processes occurring post-implantation. The host site may itself control the material through local changes in pH, ionic strength or other specific molecular interactions. The use of supramolecular assemblies of responsive polymers (e.g., shell or core cross-linking structures) can be utilized to achieve long-term structural stability. The detection motifs exhibiting more sensitive and selective responses should be further developed and incorporated into responsive polymer matrices, aimed at sensing and discriminating subtle changes in the gradients and concentrations of pH, temperature, glucose, bioactive small molecules and other biorelevant macromolecular species. The development of pH-responsive polymers is centered on systems capable of selectively detecting multiple analytes simultaneously. The challenge remains to optimize material responses and to incorporate these into medical devices to ensure that these novel smart materials reach their application potential, both *in vitro* and *in vivo*. *In vivo* uses are becoming more thoroughly investigated, with promising work towards disease therapies and targeted drug delivery.

Dual stimuli-responsive materials will give rise to technologies that combine different properties, augmenting both the specificity and efficacy of cell targeting, cell responsiveness and drug delivery. By appropriate copolymerization, cross-linking and ligand attachment the properties of smart materials can be tailored to meet the needs of specific applications. These novel strategies for producing smart materials are so far providing exciting new tools for drug delivery, neuronal and other cell manipulation and tissue engineering for regenerative medicine. However, much of the work done to date is purely experimental and of little immediate clinical benefit. The future trends must be focused on optimizing the specific and exacting requirements of these materials before their successful application for clinical therapies.

3.9 References

Akiyama, H., Ito, A., Kawabe, Y. and Kamihira, M. (2010). Genetically engineered angiogenic cell sheets using magnetic force-based gene delivery and tissue fabrication techniques. *Biomaterials*, **31**, 1251–1259.

Alemdaroğlu, C. D. Z., Celebi, N., Zor, F., Oztürk, S. and Erdoğan, D. (2006). An investigation on burn wound healing in rats with chitosan gel formulation containing epidermal growth factor. *Burns*, **32**, 319–327.

Azzaroni, O., Brown, A. A. and Huck, W. T. S. (2007). Tunable Wettability by Clicking Counterions Into Polyelectrolyte Brushes. *Advanced Materials*, **19**, 151–154.

Bajpai, A. K., Shukla, S. K., Bhanu, S. and Kankane, S. (2008). Responsive polymers in controlled drug delivery. *Progress in Polymer Science*, **33**, 1088–1118.

Barrett, T., Ravizzini, G., Choyke, P. L. and Kobayashi, H. (2009). Dendrimers in medical nanotechnology. *IEEE Engineering in Medicine and Biology Magazine: The Quarterly Magazine of the Engineering in Medicine & Biology Society*, **28**, 12–22.

Baruch-Sharon, S. and Margel, S. (2010). Synthesis and characterization of polychloromethylstyrene nanoparticles of narrow size distribution by emulsion and miniemulsion polymerization processes. *Colloid and Polymer Science*, **288**, 869–877.

Bellomo, E. G., Wyrsta, M. D., Pakstis, L., Pochan, D. J. and Deming, T. J. (2004). Stimuli-responsive polypeptide vesicles by conformation-specific assembly. *Nature Materials*, **3**, 244–248.

Bovolenta, P. and Fernaud-Espinosa, I. (2000). Nervous system proteoglycans as modulators of neurite outgrowth. *Progress in Neurobiology*, **61,** 113–132.

Braunecker, W. A. and Matyjaszewski, K. (2007). Controlled/living radical polymerization: Features, developments, and perspectives. *Progress in Polymer Science*, **32**, 93–146.

Brun-Graeppi, A. K. A. S., Richard, C., Bessodes, M., Scherman, D. and Merten, O.-W. (2011). Cell microcarriers and microcapsules of stimuli-responsive polymers. *Journal of Controlled Release*, **149**, 209–224.

Bütün, V., Sönmez, Ş., Yarligan, S., Taktak, F. F., Atay, A. and Bütün, S. (2008). Micelles and 'reverse micelles' with a novel water-soluble diblock copolymer. *Polymer*, **49**, 4057–4065.

Calderón, M., Quadir, M. A., Strumia, M. and Haag, R. (2010). Functional dendritic polymer architectures as stimuli-responsive nanocarriers. *Biochimie*, **92**, 1242–1251.

Chan, A., Orme, R. P., Fricker, R. A. and Roach, P. (2013). Remote and local control of stimuli responsive materials for therapeutic applications. *Advanced Drug Delivery Reviews*, **65**, 497–514

Chan, Y., Bulmus, V., Zareie, M. H., Byrne, F. L., Barner, L. and Kavallaris, M. (2006). Acid-cleavable polymeric core–shell particles for delivery of hydrophobic drugs. *Journal of Controlled Release*, **115**, 197–207.

Chen, T., Ferris, R., Zhang, J., Ducker, R. and Zauscher, S. (2010). Stimulus-responsive polymer brushes on surfaces: Transduction mechanisms and applications. *Progress in Polymer Science*, **35**, 94–112.

Chiappetta, D. A. and Sosnik, A. (2007). Poly(ethylene oxide)–poly(propylene oxide) block copolymer micelles as drug delivery agents: Improved hydrosolubility, stability and bioavailability of drugs. *European Journal of Pharmaceutics and Biopharmaceutics*, **66**, 303–317.

Chuang, C.-Y., Don, T.-M. and Chiu, W.-Y. (2009). Synthesis of chitosan-based thermo- and pH-responsive porous nanoparticles by temperature-dependent self-assembly method and their application in drug release. *Journal of Polymer Science Part A: Polymer Chemistry*, **47**, 5126–5136.

Coelho, J., Ferreira, P., Alves, P., Cordeiro, R., Fonseca, A., Góis, J. and Gil, M. (2010). Drug delivery systems: Advanced technologies potentially applicable in personalized treatments. *The EPMA Journal*, **1**, 164–209.

Collins, M. N. and Birkinshaw, C. (2013). Hyaluronic acid based scaffolds for tissue engineering – a review. *Carbohydrate Polymers,* **92**, 1262–1279.

Cui, F., Qian, F., Zhao, Z., Yin, L., Tang, C. and Yin, C. (2009). Preparation, characterization, and oral delivery of insulin loaded carboxylated chitosan grafted poly(methyl methacrylate) nanoparticles. *Biomacromolecules*, **10**, 1253–1258.

Dai, S., Ravi, P. and Tam, K. C. (2008). pH-Responsive polymers: Synthesis, properties and applications. *Soft Matter*, **4**, 435–449.

Dai, Y., Ma, P. A., Cheng, Z., Kang, X., Zhang, X., Hou, Z., Li, C., Yang, D., Zhai, X. and Lin, J. (2012). Up-conversion cell imaging and pH-induced thermally controlled drug release from NaYF4:Yb3+/Er3+ hydrogel core–shell hybrid microspheres. *ACS Nano*, **6**, 3327–3338.

Dash, A. and Cudworth Ii, G. (1998). Therapeutic applications of implantable drug delivery systems. *Journal of Pharmacological and Toxicological Methods*, **40**, 1–12.

Dayananda, K., Pi, B. S., Kim, B. S., Park, T. G. and Lee, D. S. (2007). Synthesis and characterization of pH/temperature-sensitive block copolymers via atom transfer radical polymerization. *Polymer*, **48**, 758–762.

Deligkaris, K., Tadele, T. S., Olthuis, W. and Van Den Berg, A. (2010). Hydrogel-based devices for biomedical applications. *Sensors and Actuators B: Chemical*, **147**, 765–774.

Du, J., Tang, Y., Lewis, A. L. and Armes, S. P. (2011). pH-sensitive biocompatible block copolymer vesicles for drug delivery. *Journal of Controlled Release*, **152**, Supplement 1, e16-e17.

Ekici, S., Ilgin, P., Butun, S. and Sahiner, N. (2011). Hyaluronic acid hydrogel particles with tunable charges as potential drug delivery devices. *Carbohydrate Polymers*, **84**, 1306–1313.

El-Sherbiny, I. M. (2010). Enhanced pH-responsive carrier system based on alginate and chemically modified carboxymethyl chitosan for oral delivery of protein drugs: Preparation and in-vitro assessment. *Carbohydrate Polymers*, **80,** 1125–1136.

Esfand, R. and Tomalia, D. A. (2001). Poly(amidoamine) (PAMAM) dendrimers: From biomimicry to drug delivery and biomedical applications. *Drug Discovery Today*, **6**, 427–436.

Felber, A. E., Dufresne, M.-H. and Leroux, J.-C. (2012). pH-sensitive vesicles, polymeric micelles, and nanospheres prepared with polycarboxylates. *Advanced Drug Delivery Reviews*, **64**, 979–992.

Gao, Q., Wang, C., Liu, H., Wang, C., Liu, X. and Tong, Z. (2009). Suspension polymerization based on inverse Pickering emulsion droplets for thermo-sensitive hybrid microcapsules with tunable supracolloidal structures. *Polymer*, **50**, 2587–2594.

Garbern, J. C., Minami, E., Stayton, P. S. and Murry, C. E. (2011). Delivery of basic fibroblast growth factor with a pH-responsive, injectable hydrogel to improve angiogenesis in infarcted myocardium. *Biomaterials*, **32**, 2407–2416.

George, M. and Abraham, T. E. (2006). Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan – a review. *Journal of Controlled Release,* **114,** 1–14.

Ghandehari, H., Kopecková, P. and Kopecek, J. (1997). In vitro degradation of pH-sensitive hydrogels containing aromatic azo bonds. *Biomaterials*, **18**, 861–872.

Gil, E. S. and Hudson, S. M. (2004). Stimuli-reponsive polymers and their bioconjugates. *Progress in Polymer Science*, **29**, 1173–1222.

Godbey, W. T. and Mikos, A. G. (2001). Recent progress in gene delivery using non-viral transfer complexes. *Journal of Controlled Release*, **72**, 115–125.

Gong, R., Li, C., Zhu, S., Zhang, Y., Du, Y. and Jiang, J. (2011). A novel pH-sensitive hydrogel based on dual crosslinked alginate/N-α-glutaric acid chitosan for oral delivery of protein. *Carbohydrate Polymers*, **85,** 869–874.

Green, J. J., Langer, R. and Anderson, D. G. (2008). A combinatorial polymer library approach yields insight into nonviral gene delivery. *Accounts of Chemical Research*, **41**, 749–759.

Gregory, A. and Stenzel, M. H. (2012a). Complex polymer architectures via RAFT polymerization: From fundamental process to extending the scope using click chemistry and nature's building blocks. *Progress in Polymer Science*, **37**, 38–105.

Gregory, A. and Stenzel, M. H. (2012b). Complex polymer architectures via RAFT polymerization: From fundamental process to extending the scope using click chemistry and nature's building blocks. *Progress in Polymer Science (Oxford)*, **37**, 38–105.

Gupta, B., Levchenko, T. S. and Torchilin, V. P. (2005). Intracellular delivery of large molecules and small particles by cell-penetrating proteins and peptides. *Advanced Drug Delivery Reviews*, **57**, 637–651.

Gupta, K. C. and Ravi Kumar, M. N. V. (2000). Drug release behavior of beads and microgranules of chitosan. *Biomaterials*, **21**, 1115–1119.

Gupta, P., Vermani, K. and Garg, S. (2002). Hydrogels: From controlled release to pH-responsive drug delivery. *Drug Discovery Today*, **7**, 569–579.

Guvendiren, M., Yang, S. and Burdick, J. A. (2009). Swelling-induced surface patterns in hydrogels with gradient crosslinking density. *Advanced Functional Materials*, **19**, 3038–3045.

Henry, S. M., EL-Sayed, M. E. H., Pirie, C. M., Hoffman, A. S. and Stayton, P. S. (2006). pH-responsive poly(styrene-alt-maleic anhydride) alkylamide copolymers for intracellular drug delivery. *Biomacromolecules*, **7**, 2407–2414.

Herber, S., Bomer, J., Olthuis, W., Bergveld, P. and Berg, A. V. (2005). A miniaturized carbon dioxide gas sensor based on sensing of pH-sensitive hydrogel swelling with a pressure sensor. *Biomedical Microdevices*, **7**, 197–204.

Ho, V. H. B., Slater, N. K. H. and Chen, R. (2011). pH-responsive endosomolytic pseudo-peptides for drug delivery to multicellular spheroids tumour models. *Biomaterials*, **32**, 2953–2958.

Hoffman, A. S. (2012). Hydrogels for biomedical applications. *Advanced Drug Delivery Reviews*, **64**, (Supplement), 18–23.

Hu, S. G., Jou, C. H. and Yang, M. C. (2003). Protein adsorption, fibroblast activity and antibacterial properties of poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) grafted with chitosan and chitooligosaccharide after immobilized with hyaluronic acid. *Biomaterials,* **24**, 2685–2693.

Hu, J. and Liu, S. (2010). Responsive Polymers for Detection and Sensing Applications: Current Status and Future Developments. *Macromolecules*, **43**, 8315–8330.

Huang, Z., Liu, S., Zhang, B., Xu, L. and Hu, X. (2012). Equilibrium and kinetics studies on the absorption of Cu(II) from the aqueous phase using a β-cyclodextrin-based adsorbent. *Carbohydrate Polymers*, **88**, 609–617.

Jeon, O., Yang, H. S., Lee, T.-J. and Kim, B.-S. (2008). Heparin-conjugated polyethylenimine for gene delivery. *Journal of Controlled Release*, **132**, 236–242.

Ji, C., Khademhosseini, A. and Dehghani, F. (2011). Enhancing cell penetration and proliferation in chitosan hydrogels for tissue engineering applications. *Biomaterials*, **32,** 9719–9729.

Jiang, X., Dausend, J., Hafner, M., Musyanovych, A., Röcker, C., Landfester, K., Mailänder, V. and Nienhaus, G. U. (2010). Specific effects of surface amines on polystyrene nanoparticles in their interactions with mesenchymal stem cells. *Biomacromolecules*, **11**, 748–753.

Joshi, A., Solanki, S., Chaudhari, R., Bahadur, D., Aslam, M. and Srivastava, R. (2011). Multifunctional alginate microspheres for biosensing, drug delivery and magnetic resonance imaging. *Acta Biomaterialia,* **7**, 3955–3963.

Kaminski, K., Zazakowny, K., Szczubiałka, K. and Nowakowska, M. (2008). pH-sensitive genipin-cross-linked chitosan microspheres for heparin removal. *Biomacromolecules*, **9**, 3127–3132.

Kang, S.-W., Lim, H.-W., Seo, S.-W., Jeon, O., Lee, M. and Kim, B.-S. (2008). Nanosphere-mediated delivery of vascular endothelial growth factor gene for therapeutic angiogenesis in mouse ischemic limbs. *Biomaterials*, **29**, 1109–1117.

Kang, S. I. and Bae, Y. H. (2002). pH-Induced solubility transition of sulfonamide-based polymers. *Journal of Controlled Release*, **80**, 145–155.

Kang, S. I. and Bae, Y. H. (2003). A sulfonamide based glucose-responsive hydrogel with covalently immobilized glucose oxidase and catalase. *Journal of Controlled Release*, **86**, 115–121.

Kasahara, H., Tanaka, E., Fukuyama, N., Sato, E., Sakamoto, H., Tabata, Y., Ando, K., Iseki, H., Shinozaki, Y., Kimura, K., Kuwabara, E., Koide, S., Nakazawa, H. and Mori, H. (2003). Biodegradable gelatin hydrogel potentiates the angiogenic effect of fibroblast growth factor 4 plasmid in rabbit hindlimb ischemia. *Journal of the American College of Cardiology*, **41**, 1056–1062.

Kataoka, K. (2004) Smart polymeric micelles as nanocarriers for gene and drug delivery. Mems, NANO and smart systems. ICMENS 2004. Proceedings on 2004 International Conference on, 25–27 August 2004, Banff, Alberta, Canada, 4–5.

Kataoka, K., Harada, A. and Nagasaki, Y. (2012). Block copolymer micelles for drug delivery: Design, characterization and biological significance. *Advanced Drug Delivery Reviews*, **64**, (Supplement), 37–48.

Katz, M. G., Swain, J. D., Tomasulo, C. E., Sumaroka, M., Fargnoli, A. and Bridges, C. R. (2011). Current strategies for myocardial gene delivery. *Journal of Molecular and Cellular Cardiology*, **50**, 766–776.

Keddie, D. J., Moad, G., Rizzardo, E. and Thang, S. H. (2012). RAFT agent design and synthesis. *Macromolecules*, **45**, 5321–5342.

Kim, B. and Peppas, N. A. (2003a). Analysis of molecular interactions in poly(methacrylic acid-g-ethylene glycol) hydrogels. *Polymer*, **44**, 3701–3707.

Kim, B. and Peppas, N. A. (2003b). In vitro release behavior and stability of insulin in complexation hydrogels as oral drug delivery carriers. *International Journal of Pharmaceutics*, **266**, 29–37.

Kim, I.-Y., Seo, S.-J., Moon, H.-S., Yoo, M.-K., Park, I.-Y., Kim, B.-C. and Cho, C.-S. (2008). Chitosan and its derivatives for tissue engineering applications. *Biotechnology Advances,* **26**, 1–21.

Kirsebom, H., Aguilar, M. R., San Roman, J., Fernandez, M., Prieto, M. A. and Bondar, B. (2007). Macroporous scaffolds based on chitosan and bioactive molecules. *Journal of Bioactive and Compatible Polymers,* **22**, 621–636.

Kong, H., Kim, E., Huang, Y.-C. and Mooney, D. (2008). Design of biodegradable hydrogel for the local and sustained delivery of angiogenic plasmid DNA. *Pharmaceutical Research*, **25**, 1230–1238.

Koul, V., Mohamed, R., Kuckling, D., Adler, H.-J. P. and Choudhary, V. (2011). Interpenetrating polymer network (IPN) nanogels based on gelatin and poly(acrylic acid) by inverse miniemulsion technique: Synthesis and characterization. *Colloids and Surfaces B: Biointerfaces*, **83**, 204–213.

Kriwet, B., Walter, E. and Kissel, T. (1998). Synthesis of bioadhesive poly(acrylic acid) nano- and microparticles using an inverse emulsion polymerization method for the entrapment of hydrophilic drug candidates. *Journal of Controlled Release*, **56**, 149–158.

Lansalot, M., Davis, T. P. and Heuts, J. P. A. (2002). RAFT miniemulsion polymerization: Influence of the structure of the RAFT agent. *Macromolecules*, **35**, 7582–7591.

Lee, C. H., Kim, J.-H., Lee, H. J., Jeon, K., Lim, H., Choi, H. Y., Lee, E.-R., Park, S. H., Park, J.-Y., Hong, S., Kim, S. and Cho, S.-G. (2011). The generation of iPS cells using non-viral magnetic nanoparticlebased transfection. *Biomaterials*, **32**, 6683–6691.

Lee, H.-I., Pietrasik, J., Sheiko, S. S. and Matyjaszewski, K. (2010). Stimuli-responsive molecular brushes. *Progress in Polymer Science*, **35**, 24–44.

Lee, J. H. and Nan, A. (2012). Combination drug delivery approaches in metastatic breast cancer. *Journal of Drug Delivery*, **2012**, 17.

Lowe, A. B. and Mccormick, C. L. (2007). Reversible addition-fragmentation chain transfer (RAFT) radical polymerization and the synthesis of water-soluble (co)polymers under homogeneous conditions in organic and aqueous media. *Progress in Polymer Science*, **32**, 283–351.

Luzinov, I., Minko, S. and Tsukruk, V. V. (2004). Adaptive and responsive surfaces through controlled reorganization of interfacial polymer layers. *Progress in Polymer Science*, **29**, 635–698.

Mahkam, M. (2007). New pH-sensitive glycopolymers for colon-specific drug delivery. *Drug Delivery*, **14**, 147–153.

Malik, N., Wiwattanapatapee, R., Klopsch, R., Lorenz, K., Frey, H., Weener, J. W., Meijer, E. W., Paulus, W. and Duncan, R. (2000). Dendrimers: relationship between structure and biocompatibility in vitro, and preliminary studies on the biodistribution of 125I-labelled polyamidoamine dendrimers in vivo. *Journal of Controlled Release*, **65**, 133–148.

Maruyama, H., Matsumoto, H., Fukuda, T. and Arai, F., Functionalized hydrogel surface patterned in a chip for local pH sensing, *Micro Electro Mechanical Systems, 2008. MEMS 2008. IEEE 21st International Conference on*, pp. 224–227.

Matyjaszewski, K. (2009). Controlled radical polymerization: state of the art in 2008. In: *Controlled/Living Radical Polymerization: Progress in ATRP*, vol. 1023, ed. American Chemical Society, pp. 3–13.

Meldrum, F. C. (2003). Calcium carbonate in biomineralisation and biomimetic chemistry. *International Materials Reviews,* **48,** 187–224.

Mespouille, L., Coulembier, O., Paneva, D., Degée, P., Rashkov, I. and Dubois, P. (2008). Synthesis of adaptative and amphiphilic polymer model conetworks by versatile combination of Atrp, Rop, and 'Click chemistry'. *Journal of Polymer Science Part A: Polymer Chemistry*, **46**, 4997–5013.

Miyata, K., Fukushima, S., Nishiyama, N., Yamasaki, Y. and Kataoka, K. (2007). PEG-based block catiomers possessing DNA anchoring and endosomal escaping functions to form polyplex micelles with improved stability and high transfection efficacy. *Journal of Controlled Release*, **122**, 252–260.

Mu, B. and Liu, P. (2012). Temperature and pH dual responsive crosslinked polymeric nanocapsules via surface-initiated atom transfer radical polymerization. *Reactive and Functional Polymers*, **72**, 983–989.

Mu, B., Liu, P., Tang, Z., Du, P. and Dong, Y. (2011). Temperature and pH dual-responsive cross-linked polymeric nanocapsules with controllable structures via surface-initiated atom transfer radical polymerization from templates. *Nanomedicine: Nanotechnology, Biology and Medicine*, **7**, 789–796.

Na, K., Lee, K. H. and Bae, Y. H. (2004). pH-sensitivity and pH-dependent interior structural change of self-assembled hydrogel nanoparticles of pullulan acetate/oligo-sulfonamide conjugate. *Journal of Controlled Release*, **97**, 513–525.

Na, K., Lee, D. H., Hwang, D. J., Park, H. S., Lee, K. H. and Bae, Y. H. (2006). pH-sensitivity and pH-dependent structural change in polymeric nanoparticles of poly(vinyl sulfadimethoxine)–deoxycholic acid conjugate. *European Polymer Journal*, **42**, 2581–2588.

Nishikawa, H., Ueno, A., Nishikawa, S., Kido, J.-I., Ohishi, M., Inoue, H. and Nagata, T. (2000). Sulfated glycosaminoglycan synthesis and its regulation by transforming growth factor-β in rat clonal dental pulp cells. *Journal of Endodontics,* **26**, 169–171.

Oishi, M. and Nagasaki, Y. (2007). Synthesis, characterization, and biomedical applications of core–shell-type stimuli-responsive nanogels – Nanogel composed of poly[2-(N,N-diethylamino)ethyl methacrylate] core and PEG tethered chains. *Reactive and Functional Polymers*, **67**, 1311–1329.

Oliveira, J. M., Salgado, A. J., Sousa, N., Mano, J. F. and Reis, R. L. (2010). Dendrimers and derivatives as a potential therapeutic tool in regenerative medicine strategies – A review. *Progress in Polymer Science*, **35**, 1163–1194.

Pack, D. W., Hoffman, A. S., Pun, S. and Stayton, P. S. (2005). Design and development of polymers for gene delivery. *Nature Reviews Drug Discovery*, **4**, 581–593.

Park, H.-J., Yang, F. and Cho, S.-W. (2012). Nonviral delivery of genetic medicine for therapeutic angiogenesis. *Advanced Drug Delivery Reviews*, **64**, 40–52.

Park, M.-R., Seo, B.-B. and Song, S.-C. (2013). Dual ionic interaction system based on polyelectrolyte complex and ionic, injectable, and thermosensitive hydrogel for sustained release of human growth hormone. *Biomaterials*, **34**, 1327–1336.

Pérez, R. A., Won, J.-E., Knowles, J. C. and Kim, H.-W. (2013). Naturally and synthetic smart composite biomaterials for tissue regeneration. *Advanced Drug Delivery Reviews*, **65**, 471–496.

Peterca, M., Percec, V., Leowanawat, P. and Bertin, A. (2011). Predicting the size and properties of dendrimersomes from the lamellar structure of their amphiphilic janus dendrimers. *Journal of the American Chemical Society*, **133**, 20507–20520.

Priya, B., Viness, P., Yahya, E. C. and Lisa, C. D. T. (2009). Stimuli-responsive polymers and their applications in drug delivery. *Biomedical Materials*, **4**, 022001.

Puppi, D., Chiellini, F., Piras, A. M. and Chiellini, E. 2010. Polymeric materials for bone and cartilage repair. *Progress in Polymer Science*, **35**, 403–440.

Qiu, Y. and Park, K. (2012). Environment-sensitive hydrogels for drug delivery. *Advanced Drug Delivery Reviews*, **64**, (Supplement), 49–60.

Ramírez, E., Burillo, S. G., Barrera-Díaz, C., Roa, G. and Bilyeu, B. (2011). Use of pH-sensitive polymer hydrogels in lead removal from aqueous solution. *Journal of Hazardous Materials*, **192**, 432–439.

Rao, J. P. and Geckeler, K. E. (2011). Polymer nanoparticles: Preparation techniques and size-control parameters. *Progress in Polymer Science*, **36**, 887–913.

Reyes-Ortega, F., Rodriguez, G., Aguilar, M. R., Lord, M., Whitelock, J., Stenzel, M. H. and SAN Roman, J. (2013a). Encapsulation of low molecular weight heparin (bemiparin) into polymeric nanoparticles obtained from cationic block copolymers: Properties and cell activity. *Journal of Materials Chemistry B*, **1**, 850–860.

Reyes-Ortega F., R. G., Aguilar, M.R., García-Sanmartín, J., Martinez, A. and San Roman, J. (2013b) Comportamiento reológico de geles biodegradables para aplicaciones en medicina regenerativa. *Órgano De La Sociedad Ibérica De Biomecánica Y Biomateriales*, **20**, **7**–19.

Risbud, M. V., Hardikar, A. A., Bhat, S. V. and Bhonde, R. R. (2000). pH-sensitive freeze-dried chitosan–polyvinyl pyrrolidone hydrogels as controlled release system for antibiotic delivery. *Journal of Controlled Release*, **68**, 23–30.

Ruan, C., Zeng, K. and Grimes, C. A. (2003). A mass-sensitive pH sensor based on a stimuli-responsive polymer. *Analytica Chimica Acta*, **497**, 123–131.

Sakaguchi, N., Kojima, C., Harada, A. and Kono, K. (2008). Preparation of pH-sensitive poly(glycidol) derivatives with varying hydrophobicities: Their ability to sensitize stable liposomes to pH. *Bioconjugate Chemistry*, **19**, 1040–1048.

Sant, V. P., Smith, D. and Leroux, J.-C. (2004). Novel pH-sensitive supramolecular assemblies for oral delivery of poorly water soluble drugs: Preparation and characterization. *Journal of Controlled Release*, **97**, 301–312.

Satish, C., Shivakumar, H. and Gupta, N. (2007). Preparation and characterization of gelatin-poly(methacrylic acid) interpenetrating polymeric network hydrogels as a ph-sensitive delivery system for glipizide. *Indian Journal of Pharmaceutical Sciences*, **69**, 64–68.

Satturwar, P., Eddine, M. N., Ravenelle, F. and Leroux, J.-C. (2007). pH-responsive polymeric micelles of poly(ethylene glycol)-b-poly(alkyl(meth)acrylate-co-methacrylic acid): Influence of the copolymer composition on self-assembling properties and release of candesartan cilexetil. *European Journal of Pharmaceutics and Biopharmaceutics*, **65**, 379–387.

Sauer, M., Streich, D. and Meier, W. (2001). pH-Sensitive Nanocontainers. *Advanced Materials*, **13**, 1649–1651.

Schork, F. J., Luo, Y., Smulders, W., Russum, J., Butté, A. and Fontenot, K. (2005). Miniemulsion Polymerization. *Polymer Particles*, **175**, 129–255.

Şenel, S., Kremer, M. J., Kaş, S., Wertz, P. W., Hıncal, A. A. and Squier, C. A. (2000). Enhancing effect of chitosan on peptide drug delivery across buccal mucosa. *Biomaterials,* **21**, 2067–2071.

Serra, L., Doménech, J. and Peppas, N. A. (2006). Drug transport mechanisms and release kinetics from molecularly designed poly(acrylic acid-g-ethylene glycol) hydrogels. *Biomaterials*, **27**, 5440–5451.

Shah, R. N., Shah, N. A., Del Rosario Lim, M. M., Hsieh, C., Nuber, G. and Stupp, S. I. (2010). Supramolecular design of self-assembling nanofibers for cartilage regeneration. *Proceedings of the National Academy of Sciences*, **107**, 3293–3298.

Shchukin, D. G., Radtchenko, I. L. and Sukhorukov, G. B. (2003). Micron-scale hollow polyelectrolyte capsules with nanosized magnetic Fe3O4 inside. *Materials Letters*, **57**, 1743–1747.

Shchukina, E. M. and Shchukin, D. G. (2012). Layer-by-layer coated emulsion microparticles as storage and delivery tool. *Current Opinion in Colloid & Interface Science*, **17**, 281–289.

Shim, W. S., Kim, J.-H., Park, H., Kim, K., Chan Kwon, I. and Lee, D. S. (2006). Biodegradability and biocompatibility of a pH- and thermo-sensitive hydrogel formed from a sulfonamide-modified poly(ε-caprolactone-co-lactide)–poly(ethylene glycol)–poly(ε-caprolactone-co-lactide) block copolymer. *Biomaterials*, **27**, 5178–5185.

Siegel, R. A., Falamarzian, M., Firestone, B. A. and Moxley, B. C. (1988). pH-controlled release from hydrophobic/polyelectrolyte copolymer hydrogels. *Journal of Controlled Release*, **8**, 179–182.

Silva, C. M., Ribeiro, A. J., Figueiredo, I. V., Gonçalves, A. R. and Veiga, F. (2006). Alginate microspheres prepared by internal gelation: development and effect on insulin stability. *International Journal of Pharmaceutics,* **311,** 1–10.

Smith, A. E., Xu, X. and Mccormick, C. L. (2010). Stimuli-responsive amphiphilic (co) polymers via RAFT polymerization. *Progress in Polymer Science*, **35**, 45–93.

Sonia, T. A. and Sharma, C. P. (2012). An overview of natural polymers for oral insulin delivery. *Drug Discovery Today*, **17,** 784–792.

Sukhishvili, S. A. (2005). Responsive polymer films and capsules via layer-by-layer assembly. *Current Opinion in Colloid & Interface Science*, **10**, 37–44.

Tan, B. H., Ravi, P. and Tam, K. C. (2006). Synthesis and characterization of novel pH-responsive polyampholyte microgels. *Macromolecular Rapid Communications*, **27**, 522–528.

Tan, B. H. and Tam, K. C. (2008). Review on the dynamics and micro-structure of pH-responsive nano-colloidal systems. *Advances in Colloid and Interface Science*, **136**, 25–44.

Tan, B. H., Tam, K. C., Lam, Y. C. and Tan, C. B. (2005). Microstructure and rheological properties of pH-responsive core–shell particles. *Polymer*, **46**, 10066–10076.

Tan, R., She, Z., Wang, M., Fang, Z., Liu, Y. and Feng, Q. (2012). Thermo-sensitive alginate-based injectable hydrogel for tissue engineering. *Carbohydrate Polymers,* **87**, 1515–1521.

Teramura, Y. and Iwata, H. (2010). Bioartificial pancreas: Microencapsulation and conformal coating of islet of Langerhans. *Advanced Drug Delivery Reviews*, **62**, 827–840.

Tian, H., Tang, Z., Zhuang, X., Chen, X. and Jing, X. (2012). Biodegradable synthetic polymers: Preparation, functionalization and biomedical application. *Progress in Polymer Science*, **37**, 237–280.

Tian, Y., Bromberg, L., Lin, S. N., Alan Hatton, T. and Tam, K. C. (2007). Complexation and release of doxorubicin from its complexes with pluronic P85-b-poly(acrylic acid) block copolymers. *Journal of Controlled Release*, **121**, 137–145.

Torres-Lugo, M. and Peppas, N. A. (2000). Transmucosal delivery systems for calcitonin: A review. *Biomaterials*, **21**, 1191–1196.

Wang, X.-Q., Dai, J.-D., Chen, Z., Zhang, T., Xia, G.-M., Nagai, T. and Zhang, Q. (2004). Bioavailability and pharmacokinetics of cyclosporine A-loaded pH-sensitive nanoparticles for oral administration. *Journal of Controlled Release*, **97**, 421–429.

Wasungu, L. and Hoekstra, D. (2006). Cationic lipids, lipoplexes and intracellular delivery of genes. *Journal of Controlled Release*, **116**, 255–264.

Wever, D. A. Z., Picchioni, F. and Broekhuis, A. A. (2011). Polymers for enhanced oil recovery: A paradigm for structure–property relationship in aqueous solution. *Progress in Polymer Science*, **36**, 1558–1628.

Winkelmann, M. and Schuchmann, H. P. (2011). Precipitation of metal oxide nanoparticles using a miniemulsion technique. *Particuology*, **9**, 502–505.

Wong, A. D., Dewit, M. A. and Gillies, E. R. (2012). Amplified release through the stimulus triggered degradation of self-immolative oligomers, dendrimers, and linear polymers. *Advanced Drug Delivery Reviews*, **64**, 1031–1045.

Wu, W., Liu, J., Cao, S., Tan, H., Li, J., Xu, F. and Zhang, X. (2011). Drug release behaviors of a pH sensitive semi-interpenetrating polymer network hydrogel composed of poly(vinyl alcohol) and star poly[2-(dimethylamino)ethyl methacrylate]. *International Journal of Pharmaceutics*, **416**, 104–109.

Xu, C., Wayland, B. B., Fryd, M., Winey, K. I. and Composto, R. J. (2006). pH-Responsive Nanostructures Assembled from Amphiphilic Block Copolymers. *Macromolecules*, **39**, 6063–6070.

Xu, F. J., Ping, Y., Ma, J., Tang, G. P., Yang, W. T., Li, J., Kang, E. T. and Neoh, K. G. (2009). Comb-shaped copolymers composed of hydroxypropyl cellulose backbones and cationic poly((2-dimethyl amino)ethyl methacrylate) side chains for gene delivery. *Bioconjugate Chemistry*, **20**, 1449–1458.

Yamasaki, E. N. and Patrickios, C. S. (2003). Group transfer polymerization in the bulk: Linear polymers and randomly cross-linked networks. *European Polymer Journal*, **39**, 609–616.

Yang, L. and Liu, H. (2013). Stimuli-responsive magnetic particles and their applications in biomedical field. *Powder Technology*, **240**, 54–65.

Yao, J., Ruan, Y., Zhai, T., Guan, J., Tang, G., Li, H. and Dai, S. (2011). ABC block copolymer as 'smart' pH-responsive carrier for intracellular delivery of hydrophobic drugs. *Polymer*, **52**, 3396–3404.

Yao, K. D., Xu, M. X., Yin, Y. J., Zhao, J. Y. and Chen, X. L. (1996). pH-sensitive chitosan/gelatin hybrid polymer network microspheres for delivery of cimetidine. *Polymer International*, **39**, 333–337.

York, A. W., Kirkland, S. E. and Mccormick, C. L. (2008). Advances in the synthesis of amphiphilic block copolymers via RAFT polymerization: Stimuli-responsive drug and gene delivery. *Advanced Drug Delivery Reviews*, **60**, 1018–1036.

Yu, B., Lowe, A. B. and Ishihara, K. (2009a). Raft synthesis and stimulus-induced self-assembly in water of copolymers based on the biocompatible monomer 2-(methacryloyloxy)ethyl phosphorylcholine. *Biomacromolecules*, **10**, 950–958.

Yu, C.-Y., Yin, B.-C., Zhang, W., Cheng, S.-X., Zhang, X.-Z. and Zhuo, R.-X. (2009b). Composite microparticle drug delivery systems based on chitosan, alginate and pectin with improved pH-sensitive drug release property. *Colloids and Surfaces B: Biointerfaces*, **68**, 245–249.

Yuan, C.-H., Lin, S.-B., Liu, B., Ke, A.-R. and Quan, Z.-L. (2009). Synthesis and characterization of poly(4-acetyl acryloyl ethyl acetate-co-acrylic acid) novel

temperature/pH-sensitive hydrogels. *Sensors and Actuators B: Chemical*, **140**, 155–161.

Yuan, W., Zhang, J., Wei, J., Zhang, C. and Ren, J. (2011). Synthesis and self-assembly of pH-responsive amphiphilic dendritic star-block terpolymer by the combination of Rop, ATRP and click chemistry. *European Polymer Journal*, **47**, 949–958.

Zhang, C., Zhang, J., Han, B., Zhao, Y. and Li, W. (2008). Synthesis of icosahedral gold particles by a simple and mild route. *Green Chemistry*, **10**, 1094–1098.

Zhang, J., Senger, B., Vautier, D., Picart, C., Schaaf, P., Voegel, J.-C. and Lavalle, P. (2005). Natural polyelectrolyte films based on layer-by layer deposition of collagen and hyaluronic acid. *Biomaterials*, **26,** 3353–3361.

Zhao, C., Nie, S., Tang, M. and Sun, S. (2011). Polymeric pH-sensitive membranes – A review. *Progress in Polymer Science*, **36**, 1499–1520.

Zhao, C., Zhuang, X., He, P., Xiao, C., He, C., Sun, J., Chen, X. and Jing, X. (2009). Synthesis of biodegradable thermo- and pH-responsive hydrogels for controlled drug release. *Polymer*, **50**, 4308–4316.

Zhao, Q. and Ni, P. (2005). Synthesis of well-defined and near narrow-distribution diblock copolymers comprising PMMA and PDMAEMA via oxyanion-initiated polymerization. *Polymer*, **46**, 3141–3148.

Zhao, Y., Yokoi, H., Tanaka, M., Kinoshita, T. and Tan, T. (2008). Self-assembled pH-responsive hydrogels composed of the RATEA16 peptide. *Biomacromolecules*, **9**, 1511–1518.

Zhou, F. and Huck, W. T. S. (2005). Three-stage switching of surface wetting using phosphate-bearing polymer brushes. *Chemical Communications*, 5999–6001.

Zhu, C., Jung, S., Luo, S., Meng, F., Zhu, X., Park, T. G. and Zhong, Z. (2010). Co-delivery of siRNA and paclitaxel into cancer cells by biodegradable cationic micelles based on PDMAEMA–PCL–PDMAEMA triblock copolymers. *Biomaterials*, **31**, 2408–2416.

Zhu, X.-M., Yuan, J., Leung, K. C.-F., Lee, S.-F., Sham, K. W. Y., Cheng, C. H. K., Au, D. W. T., Teng, G.-J., Ahuja, A. T. and Wang, Y.-X. J. (2012). Hollow superparamagnetic iron oxide nanoshells as a hydrophobic anticancer drug carrier: Intracelluar pH-dependent drug release and enhanced cytotoxicity. *Nanoscale*, **4**, 5744–5754.

3.10 Appendix: abbreviations

AA	acrylic acid
AAEA	4-acetyl acryloyl ethyl acetate
AIBN	2,2′-azoisobutyronitrile
APS	ammonium persulfate
Asp	aspartate
ATRP	atom transfer radical polymerization
BA	butyl acrylate
BMA	butyl methacrylate
CDN	candesartan cilexetil
Con A	A concavalin A

CRP	control/living radical polymerization
CS	chitosan
DMA	dodecyl methacrylate
DMAAM	*N*,*N*-dimethylacrylamide
DMAEMA	*N*,*N*-dimethylaminoethylmethacrylate
DOX	doxorubicin
EA	ethyl acrylate
ECM	extracellular matrix
EDC	1-(3-dimethylaminopropyl)-3-ethyl carbodiimide
EDGMA	ethylene glycol dimethacrylate
bFGF	basic fibroblast growth factor
EPR	enhanced permeation and retention effect
GAG	glucosaminoglycan
GI	gastrointestinal
GluOx	glucose oxidase
GTP	group transfer polymerization
HA	hyaluronic acid
HG	hydrogel
hMSC	human mesenchymal stem cells
HPG	hyperbranched poly(glycidol)
iPS	induced pluripotent stem cells
KPS	potassium persulfate
LCST	lower critical solution temperature
LMWH	low molecular weight heparin
MNP	magnetic nanoparticles
MTS	1-methoxy-1-(trimethylsiloxy)-2-methylpro-1-ene
NIPAA	*N*-isopropylacrylamide
NP	nanoparticles
O/W	oil in water
PA	peptides amphiphiles
PAA	poly(acrylic acid)
PAMAM	poly(amido amine)
PAH	poly(aromatic hydrocarbons)
PCL	poly(ε-caprolactone)
PCLA	polymerization of D,L-lactide/ε-caprolactone
PDMAEMA	poly(*N*,*N*-dimethylamino ethyl methacrylate)
PDEAEMA	poly(*N*,*N*-diethylamino ethyl methacrylate)
PEG	poly(ethylene glycol)
PEI	poly(ethylenimine)
PEM	polyelectrolyte multilayer
PMA	poly(methacrylic acid)
PMAA	poly(methyl acrylamide)

PMAETMA	poly[(2-methacryloyloxy)ethyl trimethylammonium chloride]
PMEP	poly(methacryloyl ethylene phosphate)
PMMA	poly(methyl methacrylate)
PLA	poly(lactic acid)
PLL	poly(L-lisine)
PRG	progesterone
PSS	poly(4-styrene sulfonate)
PtBA	poly(tert-butyl acrylate)
PVP	poly(vinyl pyrrolidone)
RAFT	reversible addition-fragmentation chain transfer
SI-ATRP	surface-initiated atom transfer radical polymerization
SIPN	semi-interpenetrating network
SM	sulfamethazine methacrylate
SMA	stearyl methacrylate
SMO	sulfamethazine oligomer
SRPB	stimulus-responsive polymer brushes
THF	tetrahydrofuran
VEGF	vascular endothelial growth factor
VPTT	volume phase transition temperature

4

Photo-responsive polymers: properties, synthesis and applications

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Abstract: Representative examples of recently developed polymers bearing either reversible or irreversible photosensitive chromophores are reviewed. By integrating these chromophores in different positions of polymers, various types of photo-responsive polymers including main-chain conjugated polymers, homopolymers, telechelic polymers, block copolymers, dendritic polymers, and supramolecular polymers, are obtained. Both their chemical and physical properties can be remotely controlled by light, leading to a wide range of applications such as controlled drug delivery, micropattern, responsive hydrogel, degradable materials, and soft actuators.

Key words: photo-responsive polymer, phototriggers, drug delivery, hydrogel, polymer actuators.

4.1 Introduction

Photo-responsive polymers undergo a change in their properties in response to a light stimulus.[1] Different molecular properties can be light-regulated, including conformation,[2] polarity,[3] amphiphilicity,[4] charge,[5] optical chirality,[6] conjugation,[7] etc. The light-induced molecular change is reflected in a macroscopic change of material properties like shape (i.e., contraction or bending), wettability, solubility, optical properties, conductivity, adhesion and so on. Light control possesses intrinsic advantages compared to temperature, pH, electric, and magnetic stimuli as it: (i) employs non-contact and remote application (ii) can be easily dosed in order to tune the strength of the response, and (iii) allows accurate temporal and positional resolution of the response. The functionality and, ultimately, the application potential of such a polymer are mainly determined by three parameters: (i) the magnitude of the property change after light triggering, (ii) the rate at which this change occurs, and (iii) the reversibility of the process. In general, an ideal responsive polymer is one that exhibits instantaneous and drastic property variation upon light exposure. Depending on the application, a modulation

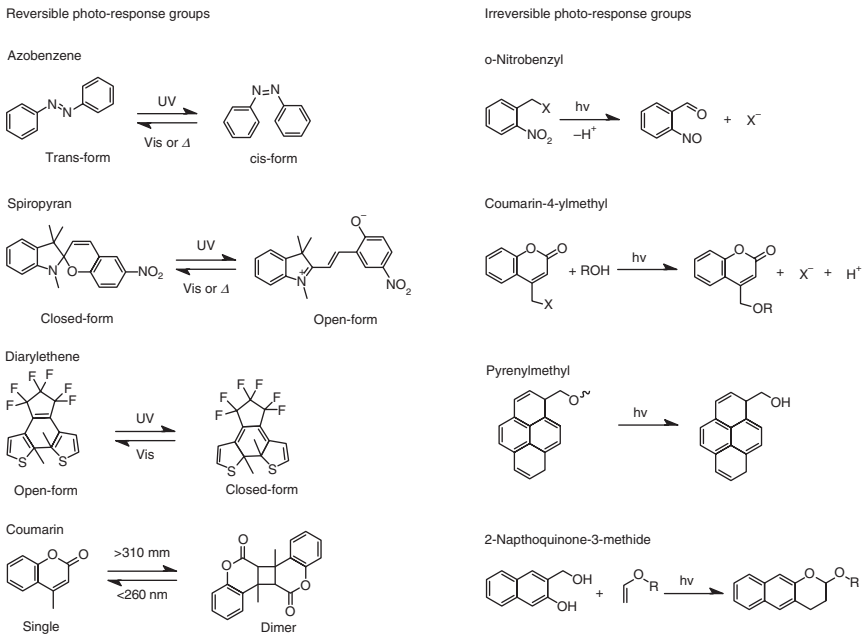
of the response with the light intensity or a reversible property change may also be advantageous.

In order to obtain photo-responsive polymers, a photo-response functional group (chromophore) needs to be incorporated into the polymer chain. Depending on the type of chromophore used, the response can be reversible or irreversible. Reversible systems can alternate material properties in two photostationary states and are used as switches. Reversibility is important in many applications such as information storage,[8] artificial muscles,[9] and actuators. Irreversible chromophores are mainly applied to photodegradable materials and used in systems for drug delivery. The advantage of irreversible chromophores is the possibility of 100% photoconversion, since no equilibrium between two states is involved. This leads to effective release of drugs[10] or to drastic decrease of the molecular weight in applications requiring degradation.[11]

Different aspects of photo-responsive polymers have recently been reviewed: Schumers *et al.* summarized reported work on photo-responsive block copolymers;[12] Zhao reported their light-induced self-assembly;[10,13] Theato and coworkers reviewed photosensitive polymers containing photoremovable groups;[14] Ikeda and coworkers described photo-responsive liquid crystalline polymers and their application as actuators;[15] Heinze and coworkers reviewed photosensitive polysaccharides.[16]Al-Malaika *et al*. described photodegradable systems[17] and Pasparakis *et al*. reviewed their applications in biotechnology.[11] Biomedical applications of photosensitive systems were reviewed by Evans and coworkers.[18] Very recently, we highlighted the functionality and application of a photolabile polymer at various surfaces.[19] In this review we focus on representative examples of recently developed polymer systems incorporating photosensitive groups excluding blends[20] and self-assembled[21] photosensitive polymers which have been recently reviewed elsewhere.[15a,22] We introduce the main types of chromophores and photo-responsive polymers and their properties in Sections 4.2 and 4.3. Special attention is paid to supramolecular polymers, being one of most relevant developments in recent years. The main applications of these systems are described in Section 4.4, including controlled drug delivery, patterned thin films of hydrogels and polymer brushes, photodegradable materials, and liquid crystal actuators. Finally, we give our critical view of the field and its future development.

4.2 Chromophores and their light-induced molecular response

Chromophores can be classified in two categories: reversible and irreversible. Reversible chromophores, often named molecular switches, undergo



4.1 Typical examples of reversible and irreversible photo-response groups.

a reversible isomerization upon light excitation at a specific wavelength. The photochromic interconversion between isomeric forms allows switching the properties of the polymer material by irradiation at two different wavelengths. Figure 4.1 presents some examples. Azobenzene alternates between planar *trans*-form and bent *cis*-form via light-induced isomerization of the –N=N– bond.[23] When coupled to a polymer chain, azobenzene has switching of hydrophility,[13] chirality,[24] optical properties,[25] and coordinative interaction[26] enabled in polymer materials. Spiropyran changes from an unconjugated spiroheterocycle to a charged planar merocyanine (MC) form with extended conjugation.[27] The light-induced change of a neutral to a charged system has been applied to control wettability,[28] vesicle dissociation,[29] molecular recognition,[30] polymer chain solubility,[31] and ion penetration.[32] Diarylethene exists as either anti-parallel or parallel rotamers. Under light exposure the anti-parallel rotamer undergoes closing of the six-membered ring within its core.[33] When attached to a polymer chain, cyclation can induce an extension of conjugation structure and rigidization. This leads to a change in the photoelectric properties of the polymer, that is, oxidation properties of polythiophene, conductivity of polyfluorene or fluorescence quantum efficiency of a photochromic system.[34] These three examples involve light-induced intramolecular transitions. Coumarin

derivatives undergo reversible intermolecular dimerization to form thermally stable and colorless isomers in response to light.[35] Dimerization has been applied to adjust the lower critical solution temperature (LCST) of polymers, or to stabilize polymersomes by intramolecular or intermolecular cross-linking.

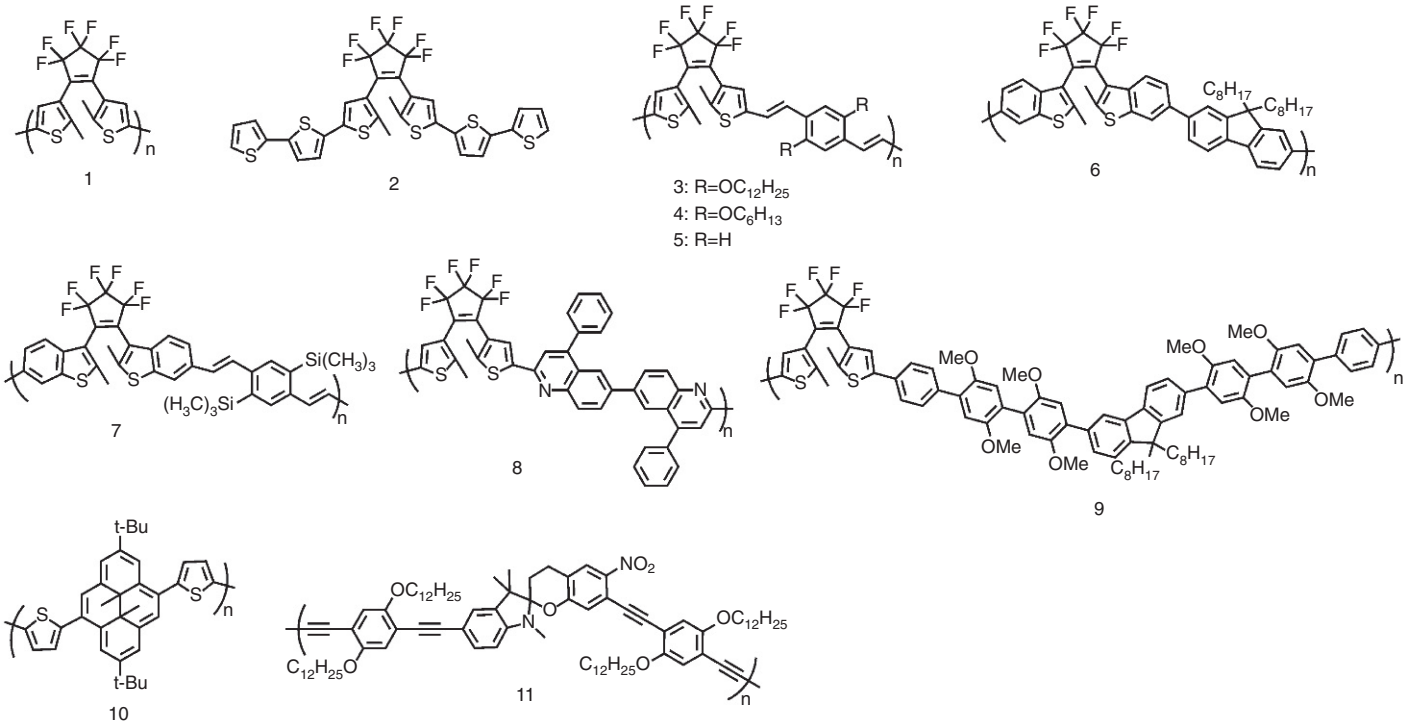
Typical examples of irreversible chromophores include photolabile protecting groups (*o*-nitrobenzyl and coumarin-4-ylmethyl derivatives), pyrenylmethyl and 2-napthoquinone-3-methides.[10,36] Photolabile groups are cleaved from the polymer chain upon light exposure. Depending on the position in the chain where the chromophore has been inserted, different light-induced molecular processes can occur: charge generation in side groups,[37] depolymerization and chain shortening,[14,38] activation of catalyst and 'click' reactant, formation of active groups, etc.[39] *o*-Nitrobenzyl derivatives undergo light-induced intramolecular oxidation resulting in the released (uncaged) functionality and a nitrosocarbonyl by-product, while coumarin-4-ylmethyl leaves a solvent-trapped coumarin by-product.[40] 2-naphthoquinone-3-methides generate a highly reactive radical which can selectively react with vinyl compounds incorporating an electron-donating group (e.g., oxygen) via very rapid Diels–Alder addition reaction, resulting in the coupling of two species.[36] Incorporating this group on polymer side chains enables light-induced reactivity which is useful in photolithography.

4.3 Key types and properties of photo-responsive polymers

From the viewpoint of chemical structure, several key types of photo-responsive polymers were collected.

4.3.1 Main-chain photochromic conjugated polymers

Photosensitive groups able to switch between a conjugated and a non-conjugated structure (e.g., diarylethenes and spiropyranares) can be introduced in the backbone of a conjugated polymer chain and applied to switch the optoelectronic properties of the material.[34] The first example of a photochromic polymer with a diarylethene-based backbone (**1**, Fig. 4.2) was reported in 1999.[41] In the diarylethene open form, it exhibited an absorbance maximum, λ_{max}, at 320 nm. Upon irradiation with UV light at 313 nm, the open form polymer was converted to the closed form which shifted the λ_{max} to > 600 nm. The most interesting property of this system was the high quantum yield experimentally obtained for the photoconversion (86%), much higher than the 50% value that is predicted in theory taking into account the coexistence of the two conformations in equilibrium. The authors attributed the experimental high quantum

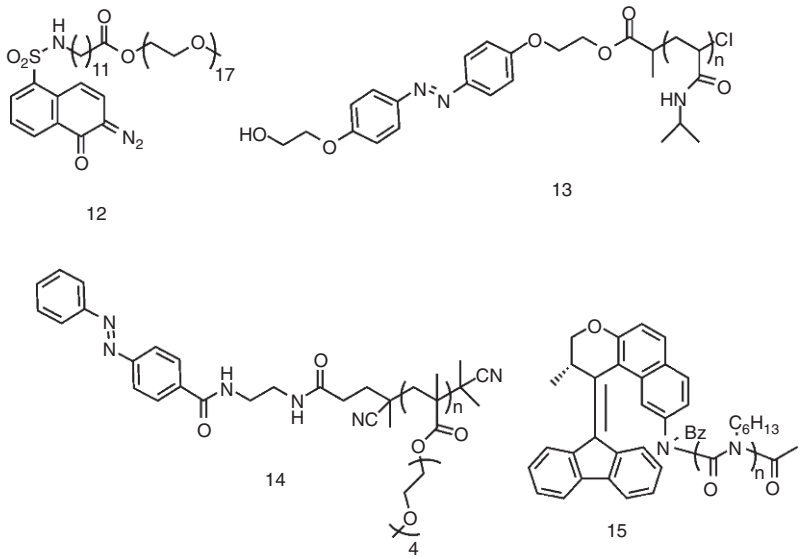


4.2 Polymers including reversible photosensitive groups in the main chain.

yield to the stabilization of the active conformation in the polymer structure as a consequence of collective conrotatory motions along the main chain. The switch between closed and open forms also enabled light regulation of the polymer electrochemical response: the closed form of **1** can undergo a reversible redox process while the open form decomposes during the redox process. This allowed a photo-gated electro-switch. This property has been demonstrated in the diarylethene-based oligothiophene **2**.[42] **2** was polymerized via electrochemical oxidation. In the presence of light only the open form was obtained, although both open and closed forms can undergo a reversible redox reaction.[42] The possibility of a photoregulated polymerization allowed the authors to deposit a pattern of the conjugated polymer on indium tin oxide (ITO). These results represent an important development for the manufacture of organic electronic devices, though no data on the photoswitching ability of the polymeric film were reported. Following this pioneering work, two other dithienylethene-based polymers (**3, 4**) were synthesized via Horner or Wittig reactions.[43] The inclusion of long alkyl chains in the polymer architecture made these systems soluble in tetrahydrofuran (THF) and facilitated the synthesis of polymers with higher molecular weights (Mn 11 600 for **3** and Mn 2702 for **5**).

In 1999 a different photochromic polymer with a diarylethene-based backbone (**6**) was obtained via Suzuki coupling of dioctylfluorene and diarylethene.[44] The resulting polymer had a photocontrollable electrical conductivity: 5.3×10^{-13} S cm^{-1} in open form and 1.2×10^{-12} S cm^{-1} in closed-ring form. The higher conductivity of the closed-ring form was attributed to the extended conjugation pathway throughout the polymer backbone. Substitution of the dioctylfluorene by trimethylsilyl substituted phenylene vinylene increased the conductivity of the resulting polymer to 3×10^{-9} (open) and 2.5×10^{-8} (closed) S cm^{-1} in polymer **7**.[45] Polymers **8** and **9** were also reported to have a photoswitchable electrical conductivity.[46]

In principle, any photochromic switching unit can be conjugated into a π-electron polymer to allow light-induced changes in the conductivity and optoelectronic properties. Dimethyldihydropyrene, for instance, was introduced into polymer **10** via Suzuki cross-coupling.[47] The closed form of polymer **10** allows conjugation through the switching core, while the open form has a localized electronic structure. A conductive polymer film was prepared from polymer **10** but solid state switching could not be observed, presumably due to a slow switching speed. In polymer **11,** containing the photochromic switch spirobenzopyran, an absorption band at > 500 nm appeared upon irradiation at 365 nm due to the formation of highly conjugated MC.[48]



4.3 Polymers containing photosensitive groups on terminal.

4.3.2 Polymers with photo-responsive terminal groups

Single photochromic groups attached to the ω-end of a polymer chain have also been used to control the properties of the polymer chains.[49] Figure 4.3 presents some examples. The first reported example was polymer **12** containing 2-diazo-1,2-naphthoquinone, a chromophoric unit which can undergo Wolff rearrangement to form changed hydrophilic 3-indenecarboxylates.[50] Amphiphilic **12** can form micelles embedding dye molecules in aqueous solution. Upon irradiation at 350 nm, the change in the hydrophilicity of the terminal group causes dissociation of the aggregates and release of the encapsulated dye. In a similar approach, polymer **13** was prepared by atom transfer radical polymerization (ATRP) of N-isopropylacrylamide (NIPAM) using an azobenzene derivative substituted with a 2-chloropropionyl group as an initiator.[51] Due to the differences in hydrophilicity between the *cis* (more hydrophilic) and the *trans* isomers, a cloud point shift from 32 to 34°C was induced by switching from *trans* to the *cis* isomer upon exposure at 365 nm. A similar effect was observed in poly(oligo(ethylene glycol) methyl ether methacrylate) with a single azobenzene end-group (**14** in Fig. 4.3).[49,52] Similar polymers modified at both ends with azobenzene showed a LCST difference up to 4.3°C between irradiated and nonirradiated solutions.[52] It is worth mentioning that this strategy is effective only with low molecular weight polymer chains, and the end-group effects vanish with increasing molecular weight.

Helical-shaped bulky alkenes are chiroptical switches and motors that can switch their helical sense in response to light.[8] When attached as end-groups of a helical polymer chain (e.g., poly(n-hexyl isocyanate), PHIC as represented in **15**), they can alternate molecular chirality by light-driven rotation.[53] PHIC without a chiral unit adopts an equal ratio of right- and left-handed helical conformation. Addition of chiral end-groups induces the polymer chain to adopt a preferred helical sense.[54] Switching of the helical sense of the terminal group enables reversible control of the preferred helical sense even in the liquid crystalline state.

4.3.3 Side-chain photochromic polymers

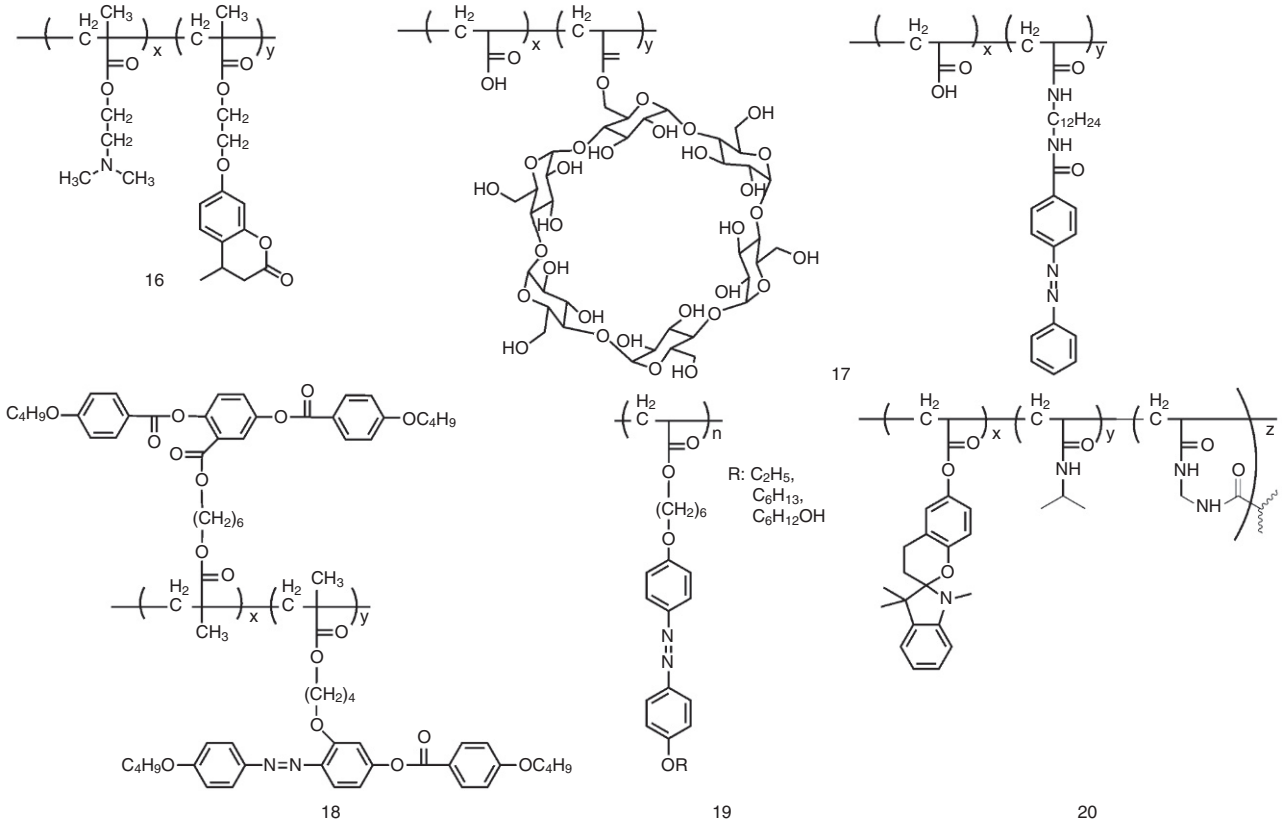
Figure 4.4 presents recent examples of polymers with reversible photochromic groups incorporated in the side chains. In copolymer **16** a dimethylaminoethyl methacrylate chain was copolymerized with coumarin-based methylacrylic monomer.[55] The LCST of a diluted solution of the copolymer polymer could be modulated between 35 and 65°C by photocontrolled intramolecular dimerization of the coumarin units upon exposure at 310 nm. The light-triggered formation of chain loops reduced interchain entanglement and caused an increase in the cloud point. Azobenzene- and spiropyran-containing copolymers with light-regulated LCSTs have also been reported.[56] The LCST of spiropyran-containing copolymers increased when the chromophore was switched from hydrophobic neutral form to the hydrophilic charged state. The mechanism of azobenzene regulation is less straightforward. In general, bent *cis*-azobenzene (with a dipole moment of ~4.4 D according to a density functional theory calculation) is more hydrophilic than the *trans*-azobenzene (0 D) and, therefore, the cloud point of the polymer increases when switching to the *cis* isomer. However, the opposite effect was observed in a copolymer of N,N′-dimethylacrylamide and azobenzyl methacrylate. The *cis*-form seems to interact with the neighboring N,N′-dimethylacrylamide unit and this interaction lowers the LCST.

The copolymer mixture **17** exploits the host–guest interaction of azobenzene and cyclodextrins (CDs) as responsive engines to induce light-control assembly.[57] *trans*-Azobenzene can be selectively encapsulated by CD and, therefore extend polymer molecular weight via intermolecular cross-linking. Upon UV exposure, the azobenzene unit undergoes *trans-cis* isomerization and is released from the α-CD site. This leads to an effective decrease in the molecular weight and, consequently, to a decrease of the viscosity of the solution. A similar strategy was used to control sol–gel transition.[58]

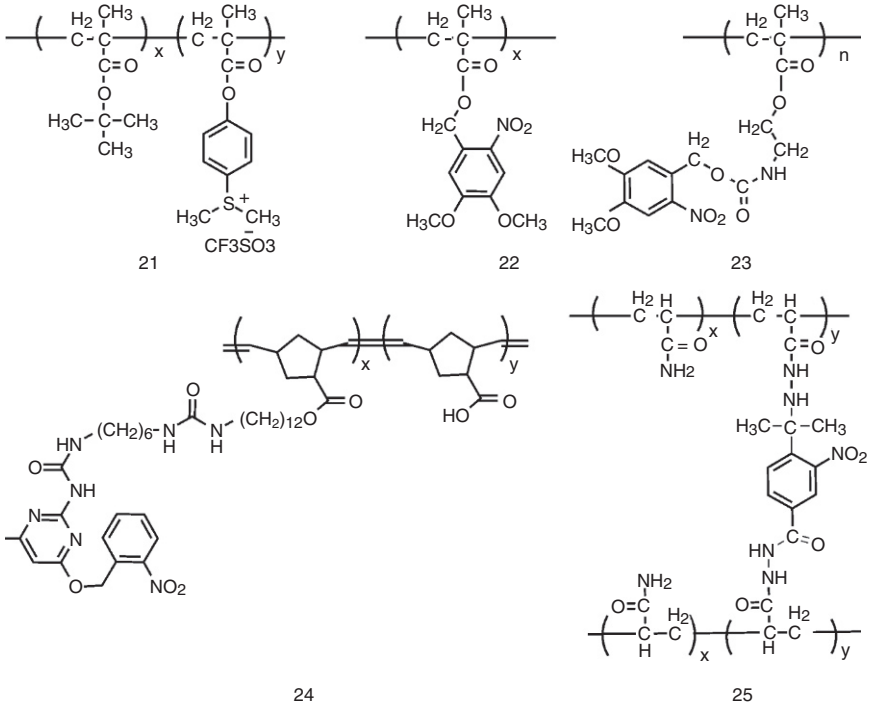
Liquid crystal polymers containing azobenzene groups in the side chains have also been reported. Polymers **18** and **19** are significant examples in which light-triggered conformational changes of the azobenzene units resulted in nematic/smectic-isotropic phase transitions.[15a,59] These systems will be discussed in detail in Section 4.3. Isomerization of spiropyran moieties (**20** in Fig. 4.4) introduced in the side chain have also been used to modulate polymer solubility in an aqueous environment[60] and we will further discuss this below.

Figure 4.5 shows some examples of polymer systems carrying irreversible photochromic groups in side chains. Polymer **21** bears a dimethylphenylsulfonium triflatecan unit as photoacid generator which undergoes homolytic cleavage followed by hydrogen abstraction and rearrangement to generate triflic acid after exposure with UV light of 254 nm.[37c] The resulting strong acid catalyzes the hydrolysis of neighbouring t-butyl esters and leads to the formation of poly(methacrylic acid) (PMAA). Thin films of this polymer show a light-induced wettability change. In a different approach, photocleavable units attached to ionizable carboxylic or amine groups were exploited to change the solubility and wettability of polymer brushes (**22**, **23**).[37b,61] Polymer **22** presents one example where the 4,5-dimethoxy-2-nitrobenzyl (DMNP) photocleavable protecting group is attached to the side-chain carboxylic groups of a PMAA chain.[37a] Light irradiation removed the DMNP group and released free carboxylic groups. When a surface covered with these polymer brushes was irradiated through a mask, a surface pattern with zones of different wettabilities was generated. Regulation of the exposure dose allowed the development of different wetting states as a consequence of different photoconversion degrees.[61] Polymer **23** with an DMNP-protected amine group has been applied to allow selective light control of the ionic permeation when incorporated into the pores of membranes.[37b,62]

Polymer **24** represents a strategy to photoregulate the formation of supramolecular polymers by attaching a caged 2-ureido-4-pyrimidone (UPy) unit in the side chain.[63] UPy can dimerize via self-complementary quadruple H-bonding with high affinity.[64] In polymer **24**, UPy units were modified by reaction with the photolabile *o*-nitrobenzenyl. This modification inactivated the H-bonding acceptor. Irradiation of a dilute solution of **24** removed the cage and induced intramolecular cross-linking by H-bonding, resulting in the formation of single molecular nanoparticles. In contrast, the *o*-nitrobenzyl chromophore in polymer **25** was designed to be a photodegradable cross-linker, which allowed UV-mediated depolymerization. This system underwent softening by 20–30% upon irradiation at a dose tolerated by living cells.[65]



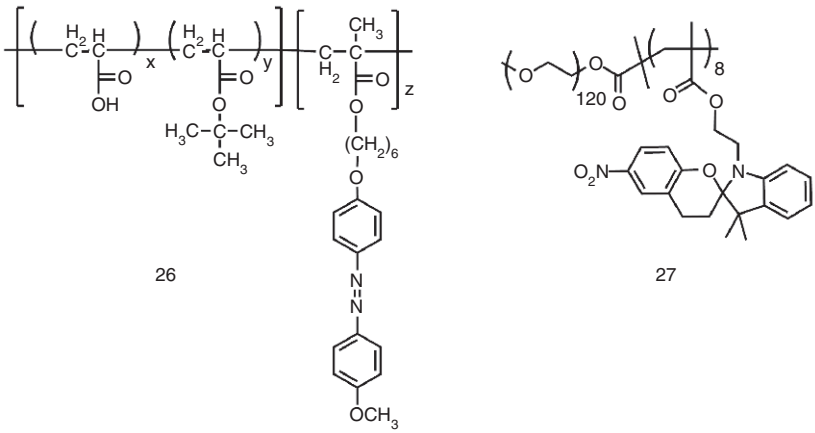
4.4 Polymeric systems with reversible photosensitive groups in the side chains.



4.5 Polymers with photolabile groups on side chains.

4.3.4 Side-chain photochromic block copolymers

Photosensitive block copolymers have been intensively studied due to their self-assembling properties and drug delivery applications. Several recent reviews[10,12,14] have been published and, therefore this section reviews only very recent developments. Figure 4.6 presents diblock copolymers with reversible photo-response. Most of these systems have been developed to photocontrol micelle formation by changing the hydrophilic–hydrophobic balance in the chain. Pioneering work was carried out in Zhao's research group. They used ATRP to synthesize polymer **26**, a block copolymer containing one random poly(t-butyl acrylate-co-acrylic acid) sequence and a poly-(methacrylate) block with azobenzene chromophores in the side chains.[66] The polymer self-assembled into core–shell micelles or vesicles when the azobenzene adopted the *trans*-form, an almost symmetrical structure with a near zero dipole moment (no charge separation). Illumination of the micellar solution with UV light (360 nm) switched the *trans*-azobenzene to its *cis*-isomer with a dipole moment of ~4.4 D, which resulted in a large increase in the polarity of the hydrophobic block. As a



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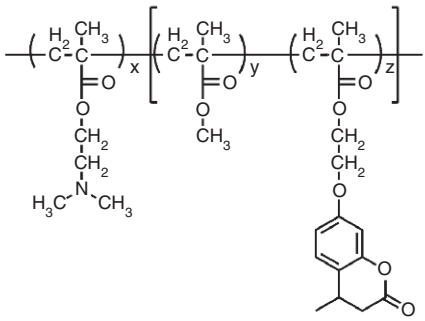

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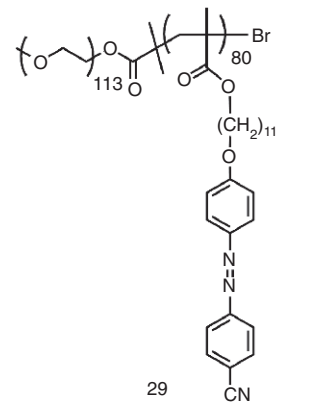

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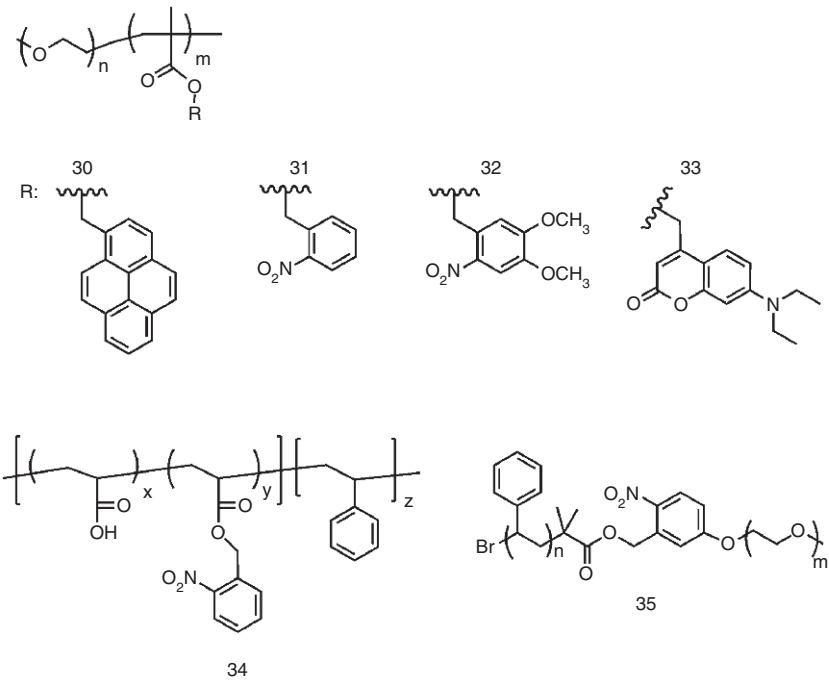
4.6 Block copolymers with reversible photosensitive groups on side chains.

result, the azobenzene-modified block was no longer hydrophobic enough to preserve the micellar association, and micelle dissociation occurred. By exposing the system to visible light (440 nm), the *trans* isomer was favoured and micelles re-formed. This approach can be applied to any photochromic molecule with isomers with different polarities, as in the case of polymer **27** consisting of a hydrophilic PEO block and a methacrylate ester block with photosensitive spiropyrane on the side chain.[29] With its amphiphilic structure, **27** self-assembled into micelles. Under UV irradiation, conversion of the hydrophobic spiropyran moieties into their hydrophilic zwitterionic MC counterparts occurred and, consequently, the micelles disassembled. Recovery of the micelles was triggered by exposure to visible light (620 nm). Polymer **28** formed micelles with reversible



4.7 Block copolymers with photolabile groups on side chains.

cross-links[67] and polymer **29** formed liquid crystalline phases with photo-switchable orientation in the solid state.[68]

Figure 4.7 exhibits examples of diblock copolymers with irreversible photo-response. Polymer **30** contains photolabile protecting groups attached to carboxylic groups in the side chains.[69] Upon UV irradiation, photosolvolysis of the pyrenylmethyl ester occurs, 1-pyrenemethanol is cleaved from the polymer chain and carboxylic acid groups are released. As a consequence, the hydrophobic block turns into a hydrophilic PMAA block. Core–shell micelles formed by **30** disappeared after irradiation with UV light at 365 nm. This design was further validated with other chromophores (polymers **31–34**).[70]

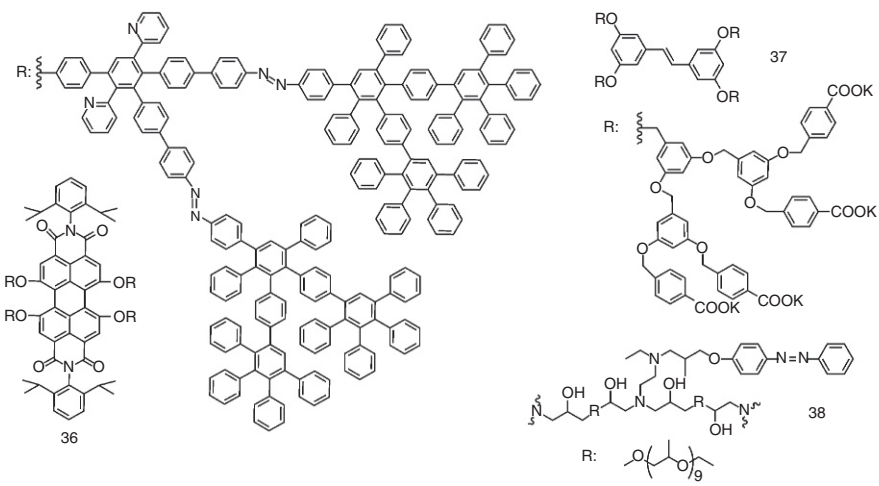
In polymer **35** two polystyrene (PS) and PEO blocks were connected with the photolabile *o*-nitrobenzyl linker.[38] Light exposure cleaved the polymer backbone and separated the hydrophobic and hydrophilic blocks. This process was successfully carried out in solution and also in solid state. Thin films of polymer **35**, annealed to obtain the vertically aligned cylindrical morphology, were exposed and washed with methanol/water after irradiation. A nanoporous PS film was obtained.[71] This kind of diblock copolymer

can be obtained either by ATRP polymerization or by copper(I)-catalyzed azide–alkyne cycloaddition of the two presynthesized blocks. The latter method is preferred if the composition of the copolymer needs to be tailored.[4d,72]

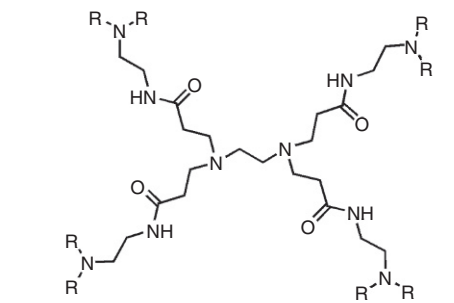
4.3.5 Photosensitive dendritic polymers

Aida and coworkers reported the first azobenzene-core dendrimer in 1997.[73] The phenyl ring on the periphery of the dendrimer can harvest the light at long wavelengths and transfer the energy into the core via a multiphoton absorption process, leading to the *trans-cis* transition of azobenzene. Reported work on light-harvesting dendrimers has recently been reviewed[74] and, therefore, we do not include it in this chapter.

Azobenzene-based dendrimers or dendrons constitute a big family.[4c,75] The chromophore can be integrated in the molecular periphery, at internal positions, or in the core, depending on the expected properties.[76] Reported examples were reviewed in 2010[75] and we cover here only relevant systems reported since then (Fig. 4.8). Dendrimer **36** contains azobenzenyl-linked polyphenylenes.[77] Upon irradiation at 365 nm, the extended six dendrons curled towards the core as a consequence of the *trans-cis* transition of azobenzenyl spacers, resulting in a highly dense and closed structure. Because of its rigidity, the dendrimer can retain guest molecules in the closed form and release them in response to light. Such photo-induced size change is also found in carbosilane dendrimers



4.8 Photosensitive dendrimer and dendritic polymers.[77,79,81]



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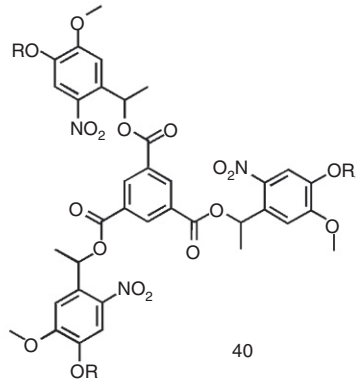

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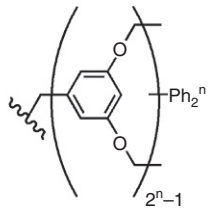

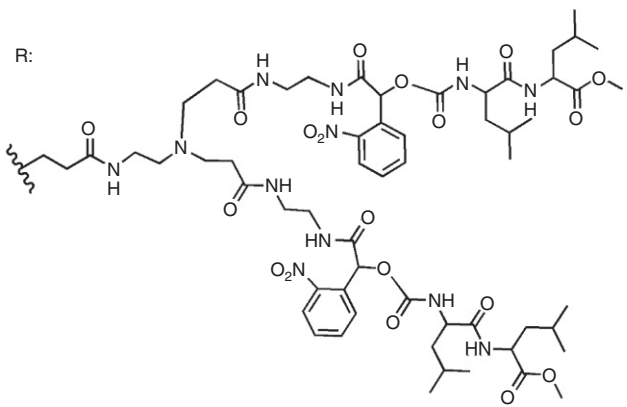

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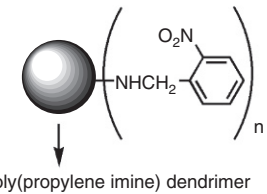


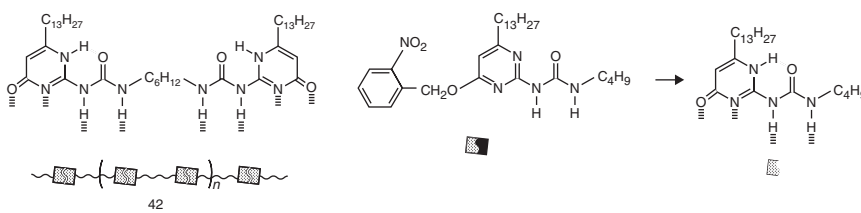
Poly(propylene imine) dendrimer

41

4.9 Dendritic polymer with irreversible photosensitive groups.

bearing 4-phenylazobenzonitrile units.[78] Dendrimer **37** is the first water-soluble dendrimer that responds to light-stimulus.[79] UV irradiation in aqueous solution induces unusual, irreversible *trans-cis* isomerization leading to 100% *cis* isomer. Interestingly, the fluorescence maximum of the stilbene core in this dendrimer shifted from 424 to 411 nm and then to 389 nm by changing peripheral dendritic structure from generation 1 to 3. This shift was attributed to the isolation of the stilbene core by the hydrophobic dendron, which decreases the interaction between the stilbene and water and, consequently, reduces the stabilization of the excited state of the stilbene core by water. Stilbene was also integrated into the branch, but the photo-responsive behaviour was not demonstrated.[80] Hyperbranched polymer **38** obtained by modification of hyperbranched poly(ether amine) with 4-phenylazophenyl glycidyl ether self-assembled in aqueous solution at 80°C into nanoparticles with 10–18 nm diameter.[81] Light exposure switched *trans*-azobenzyl to the *cis*-form which caused an increase in the LCST of the system of 5.3°C. These polymers may be good candidates to develop drug delivery systems because they can be easily synthesized and possess intrinsic 'core–shell' structures.

Irreversible photo-responsive dendritic polymers have mainly been developed for photodegradation properties.[11,82] Figure 4.9 displays three examples containing photolabile groups at core, branch, and periphery of the dendritic structures. Compound **39** was the first reported caged dendritic structure with a LeuLeuOMe unit on the periphery connected by a photolabile spacer to the core.[83] Upon irradiation, about 50% LeuLeuOMe was released. Polymer **40** contains a photolabile core which converts the original polymer into smaller dendrons by photodegradation under UV exposure.[84] Dendrimer **41** contains photolabile *o*-nitrobenzyl groups at the periphery which formed a hydrophobic rigid shell that can prevent the diffusion of encapsulated salicylic acid.[85] UV exposure cleaved the chromophore and left an amine-terminated dendrimer which



4.10 Supramolecular polymer consisting of two UPy units and monofunctional UPy unit with and without photolabile protected group. (*Source*: Adapted with permission from Reference [86].)

breaks the shell. As a consequence, the release of encapsulated molecules in the dendrimer could be significantly improved.

4.3.6 Photosensitive supramolecular polymers

In 1998, supramolecular polymers with photocontrollable molecular weights were reported for the first time (Fig. 4.10).[86] These are formed by the self-assembly of telechelic polymers terminated into UPy units. UPy can dimerize by quadruple H-bonding with high affinity (K_m of 2.2×10^6 M^{-1} in chloroform), building up long chains.[64b] A chloroform solution of this polymer (**42**) behaves like a solution of a conventional covalently linked polymer.

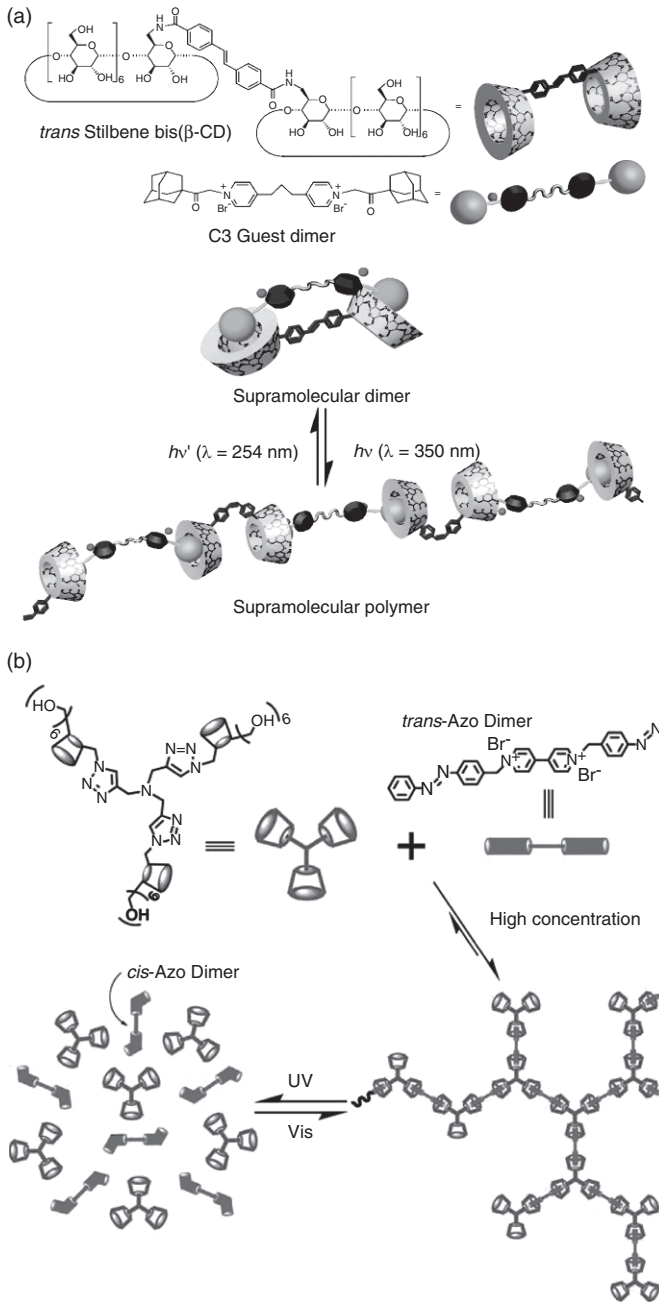
The degree of polymerization and, therefore, the viscosity, shearing effects, and viscoelastic properties are controlled by the ratio of monofunctional UPy added to the solution, which end-caps the growing chains.[64b] By protecting the UPy end-groups or the monofunctional UPy with the photolabile group *o*-nitrobenzyl, the ability of UPy to dimerize is prevented.

As a consequence, the polymerization and depolymerization processses can be tuned by irradiation with UV light. This phototriggering H-bonding strategy was applied to synthesize single molecule nanoparticles (polymer **24**) and prepare light-responsive hydrogels with self-healing ability.[87]

Figure 4.11 presents a different example of a photosensitive supramolecular polymers based on a stilbene-controlled host–guest interaction between cyclodextrin (CD) and adamantine-terminated dimers (polymer **43**).[88] The stilbene spacer undergoes a light-induced reversible *trans-cis* photoisomerization and changes the orientation and the distance between CD units. In *trans* conformation, the monomers self-assemble into dimers or short oligomers. Upon irradiation with UV light of 350 nm, stilbene is converted into its *cis* conformation and allows formation of supramolecular linear polymers with high molecular weight.

A similar host–guest interaction was exploited to prepare photo-responsive, supramolecular, hyperbranched polymer **44** using an azobenzene dimer and a β-CD trimer.[89] Light-induced *trans*-to-*cis* transition of azobenzene results in a bent conformation of the guest and disfavoured host–guest interaction with β-CD.

As a consequence, depolymerization occurs. *Cis*-to-*trans* isomerization favours polymerization. Such photoswitched association and disassociation of a non-covalent connection within the backbone has been suggested as an alternative for preparing self-healing materials.[21a,58b]



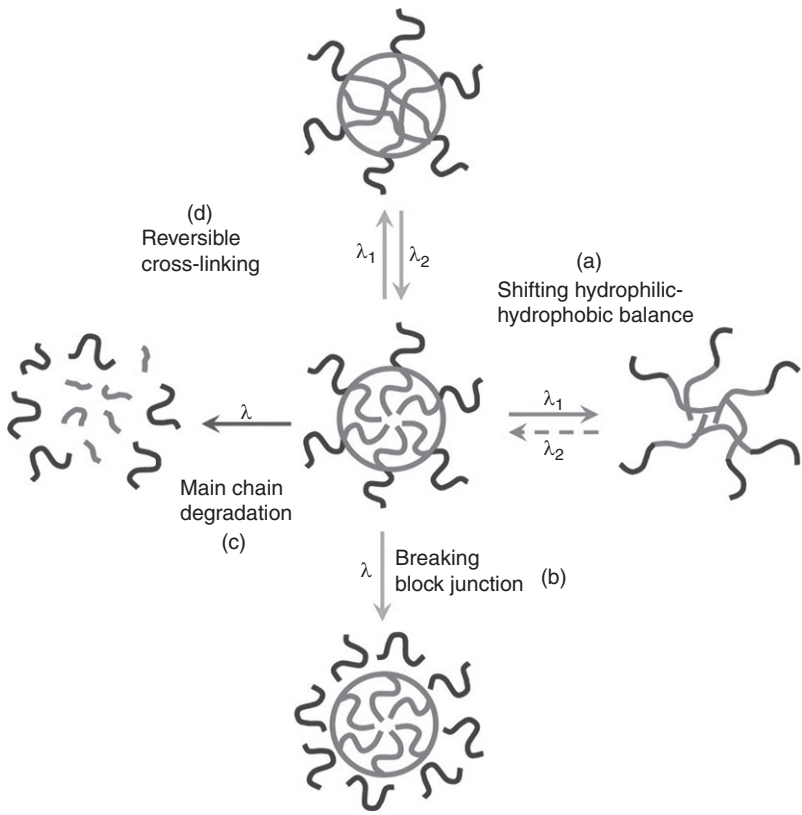
4.11 (a) Structures of stilbene bis(*β*-CD) dimer and C3 guest dimer and the photoswitched transition between dimer and polymer. (*Source*: Adapted with permission from Reference [88].) (b) Schematic representation of the photocontrolled polymerization and depolymerization of a *β*-CD3/diazo supramolecular hyperbranched polymer based on host–guest interactions. (*Source*: Adapted with permission from Reference [89].)

4.4 Applications

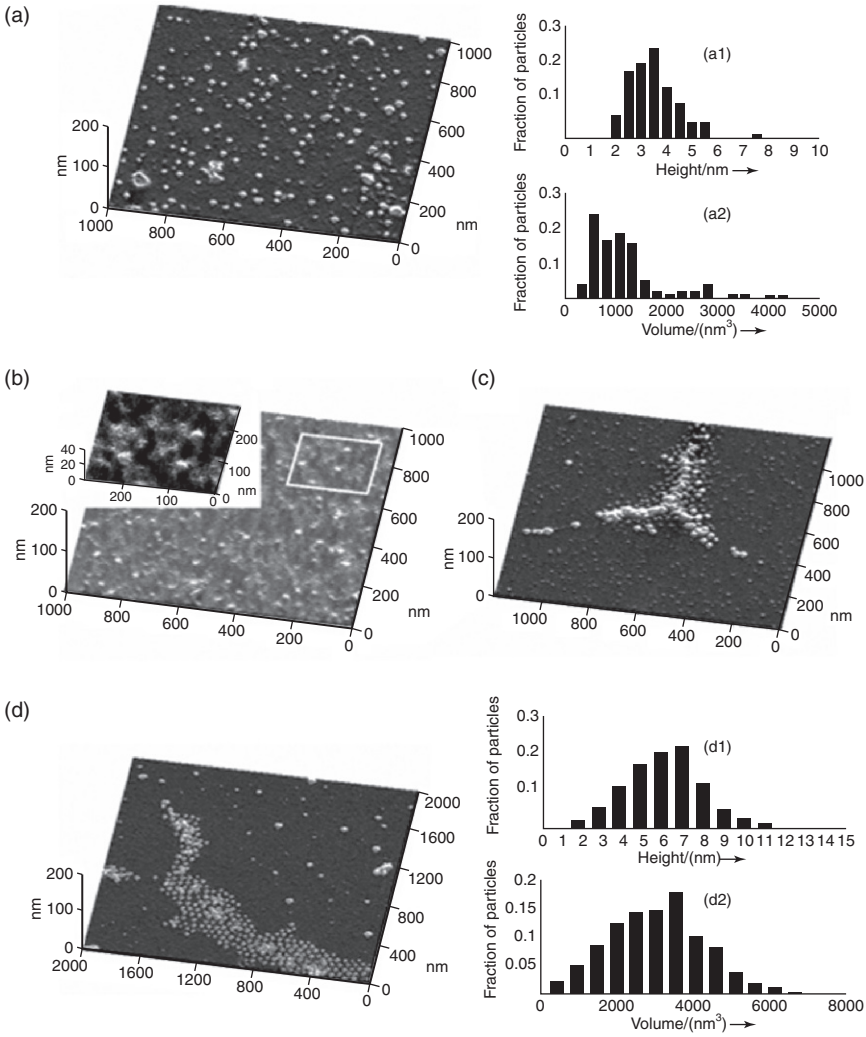
Light-induced changes at polymer level can be reflected on macroscopic level of material properties, which has been used in various application.

4.4.1 Controlled drug delivery

Polymer micelles or vesicles formed through self-assembly of photo-response block copolymers can be applied as carriers for controlled drug delivery. Figure 4.12 schematically illustrates the general mechanism for photocontrolled polymer micelles: light exposure induces solubility changes in the block modified with the photochromic group and, as a consequence, the micelles disassemble.[10,13] Figure 4.13 presents a typical example of reversible photoregulated micelles.[29] The diblock copolymer **27** self-assembles into micelles with the hydrophobic spiropyran-based block in the core.



4.12 (a–d) Schematic illustration of various types of light-responsive block copolymer micelles. (*Source*: Adapted with permission from Reference [13].)



4.13 Atomic force microscopy (AFM) images of (a) a micellar solution spin coated on mica (a1 and a2 are height and volume distributions of micellar aggregates, respectively) (b) dissociated micelles after 30 min UV exposure (365 nm). (c) reformed micelles after subsequent visible light (620 nm) exposure for 30 min, and (d) for 120 min (d1 and d2 are height and volume distributions of reformed micellar aggregates, respectively). (*Source*: Adapted with permission from Reference [29].)

Light exposure switches hydrophobic spiropyran to the charged MC form which enhances the solubility of polymer chains and, consequently, the micelles disassemble. When the micelle was loaded with a hydrophobic dye, UV exposure allowed release of the dye, which could be re-entrapped by irradiation with visible light.

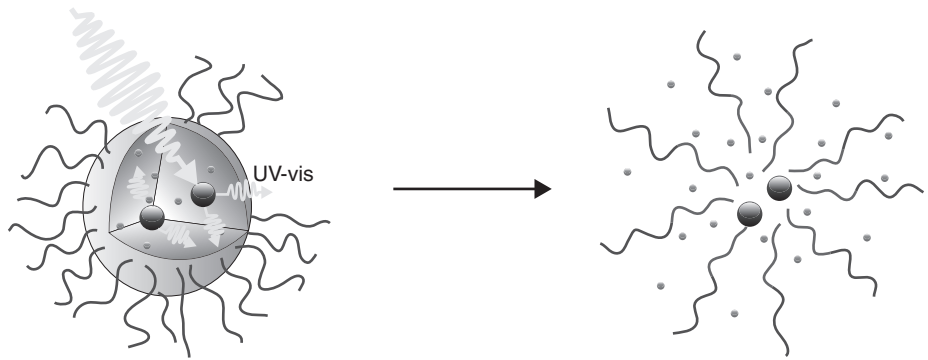
Light excitation in the near-infrared (NIR) is more convenient for drug delivery because it is able to penetrate the tissue and it does not cause cell damage. Although two-photon excitation can, in principle, extend the activation wavelength of a chromophore to the NIR region, most chromophores have low two-photon-absorption cross-sections and the photoreaction occurs with low efficiency and requires high power femtosecond pulse lasers. To overcome this limitation, lanthanide-doped upconverting nanoparticles (UCNPs) have been proposed for building NIR light-response micelles (Fig. 4.14).[90] UCNPs can absorb NIR light and then convert it into higher energy photons in the UV and visible regions which is absorbed by the photochromic moieties of polymer **30** in the core-forming block. As a consequence of the photocleavage reaction, the core becomes hydrophilic and the micelles dissociate and then release their payload.

4.4.2 Functional micropatterns

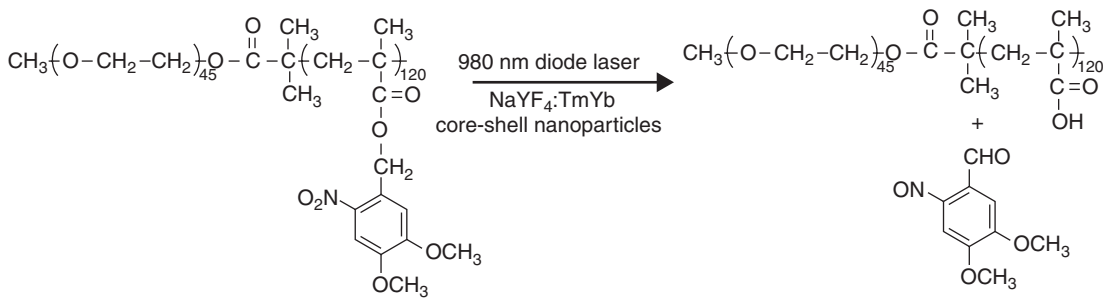
Site-selective exposure of thin films of photosensitive polymers using masks or scanning lasers can be applied to make functional patterns onto substrates, like the examples presented in Fig. 4.15.[28,91] Thin films of the main-chain conjugated polymer **7** spin coated on an electrode generate a colour conductive pattern when irradiated through a mask with parallel micrometric stripes (Fig. 4.15a). The dark areas represent the masked, highly conductive region. The bright areas correspond to the light-exposed, resistive region where diarylethene adopts its open form with lower conductivity.[45] Azobenzene-based polymers have been repeatedly used for creating surface microreliefs or gratings when an interfering laser is used for illumination.[92] Figure 4.15b presents atomic force microscopy (AFM) images of the surface relief grating formed on films of epoxy-based polymers containing azobenzyl groups at side chains after irradiation at 488 nm. Light exposure with high energy interfering laser induces mass transport in the polymeric film, which involves the scission of covalent bonds and mass transition. The mechanism of the laser-induced periodic surface structure is still unclear. Thin films of 4,5-dimethoxy-2-nitrobenzyl caging PMAA (**22)** generated a chemical pattern with regions of different wettability upon light exposure (Fig. 4.15c). The light-induced release of the *o*-nitrobenzyl photolabile protecting group from the polymer structure generates a polyelectrolyte and, consequently, makes exposed regions hydrophilic and pH-sensitive.[61] In contact with water or in humid atmospheres, the exposed regions can take up water and swell, generating a surface relief with a pH-tunable height difference between irradiated and nonirradiated regions.

Photoreactive chromophores incorporated into polymer films can be used for inducing site-specific surface reactions and generation of chemical patterns. The chromophore 2-naphthoquinone-3-methide generates highly reactive radicals upon exposure, which can selectively react with vinyl

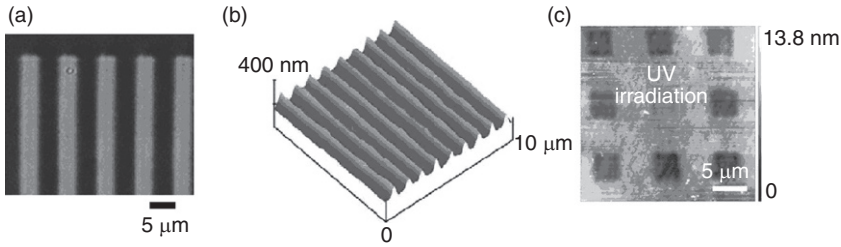
(a) NIR (980 nm)



(b)



4.14 (a,b) Photosensitive micelles that encapsulate upconversion nanoparticles (UCNPs) and allow excitation in the NIR. (*Source*: Adapted with permission from Reference [90].)

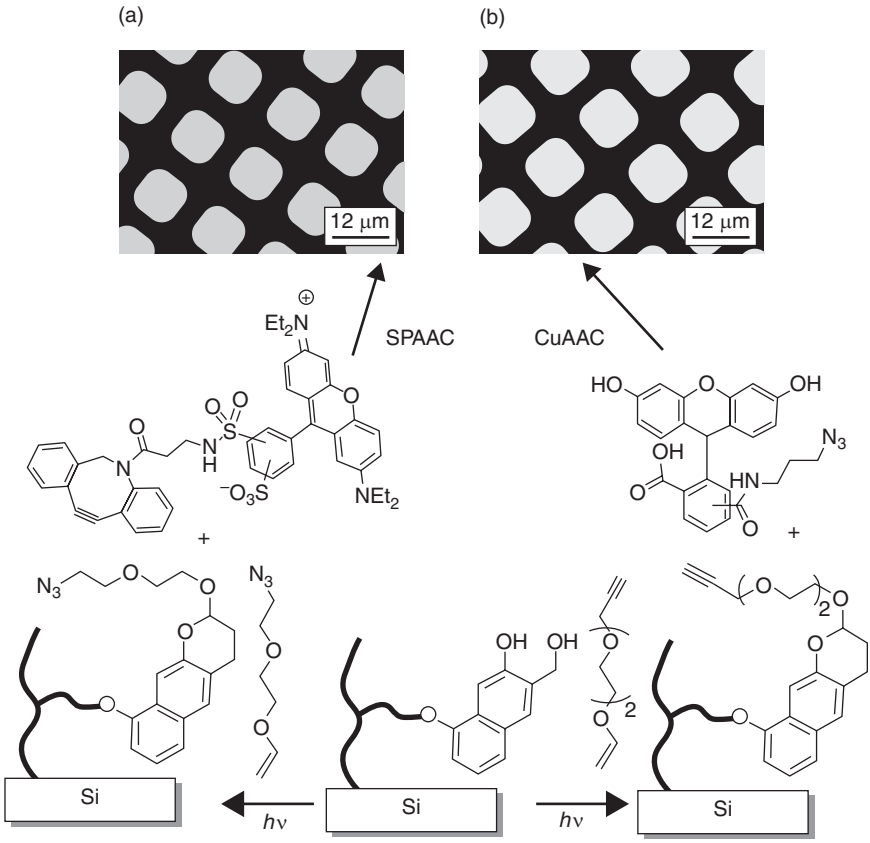


4.15 Patterns of polymer 7 (a), azobenzenyl polymer (b) and polymer 22 (c). (*Source*: Adapted with permission from References [45], [92] and [61], respectively.)

groups with electron-donating substituents, or turn back to their ground state and regenerate the photochemical precursor (Fig. 4.16).[36] This chromophore has been incorporated in the side chain of a poly(N-hydroxysuccinimidyl 4-vinylbenzoate) backbone and used for the surface immobilization of different species. An azide and an alkyne-terminated vinyl ether were photopatterned onto the polymer surface and then reacted with an alkyne or azide-terminated fluorophore using the azide–alkyne click reaction.[36d] A fluorescent pattern was obtained. This simple method can be extended to attach any molecule or biomolecule to the surface with high yield and good selectivity.

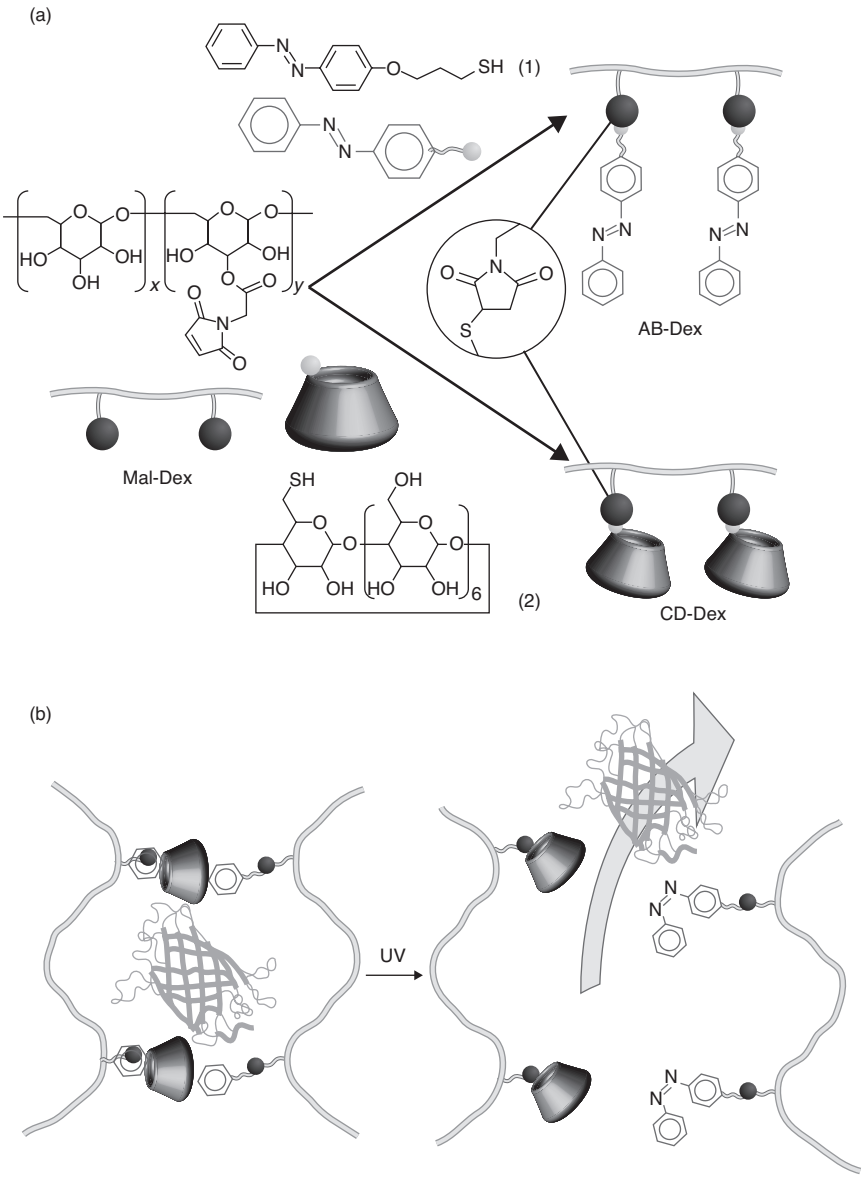
4.4.3 Responsive hydrogels

Stimulus-responsive polymeric hydrogels are useful materials that find application in drug/gene delivery, photography, paints/coatings, scaffolds for tissue engineered prostheses, biosensors, or actuators.[93] In most of these cases, the stimulus causes a molecular change (ionization, cross-linking) that affects the swelling degree of the hydrogel. In 1967, Lovrien suggested a strategy to prepare photo-response hydrogels with photochromic dyes[94] and this was first experimentally realized by Van der Veen and Prins in 1971.[95] A poly(2-hydroxyethyl methacrylate) hydrogel was mixed with sulfonated bisazostilbene dye, which decreased the hydrophilicity of the polymer chain by physical bonding in *trans*-form.[95] However, the first relevant example of light-responsive polymeric hydrogel was not reported until 1984.[96] The chromophore triphenylmethane leuco was introduced into polyacrylamide or poly(N-isopropylacrylamide) (PNIPAM) hydrogels to obtain a photoregulated swelling and shrinkage due to the reversible, light-induced ionization of the chromophore.[97] Recently, the complexes of azobenzene derivatives and CD have been integrated into hydrogels as responsive engines to light-induced swelling changes. For example, α-CD, a

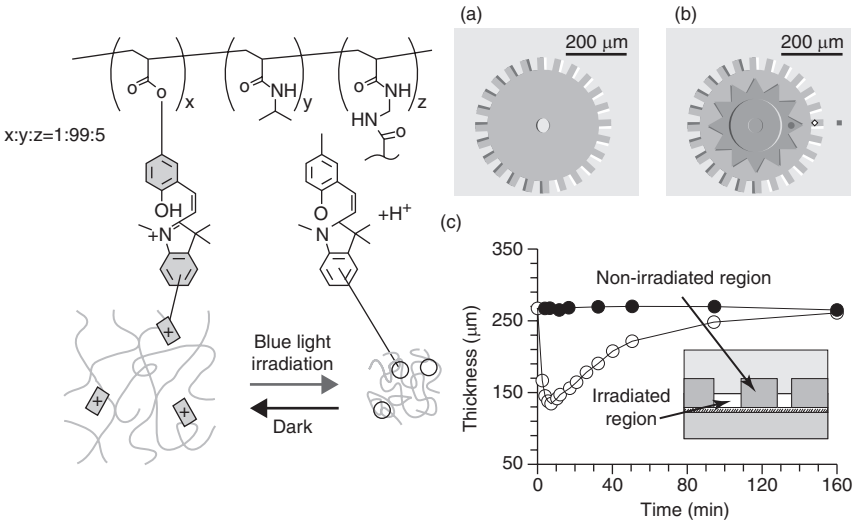


4.16 Generation of chemical patterns with photoreactive polymers: photo-Diels–Alder surface anchoring followed by azide–alkyne click reaction to immobilize fluorescent dyes. (a) SPAAC: strain-promoted azide–alkyne cycloaddition and (b) CuAAC: Cu(I)-catalyzed azide–alkyne cycloaddition. (*Source*: Adapted with permission from Reference [36d].)

dodecyl-modified poly(acrylic acid), and 4,4′-azodibenzoic acid have been combined to generate a hydrogel with light-controlled gel–sol transition. In the *trans*-form, this system does not form a gel because azobenzene has great higher affinity with CD than the dodecyl group and consumes most of the CD by forming azobenzene/CD complexes. Under irradiation, the azobenzene derivative undergoes *trans*-to-*cis* isomerization; it is released from the α-CD site and allows self-assembly of the dodecyl groups and transition to the gel form.[58a] A similar strategy was adapted to dextran hydrogels and applied to control the release of a protein (Fig. 4.17).[98] The host–guest molecules, azobenzenyl and β-CD, were attached to the dextran backbone via thiolene-click reactions. In the *trans*-form, the azobenzenyl group forms the host–guest complex with the β-CD and this results



4.17 (a) Modification of dextran with azobenzene and cyclodextrin through the thiol–maleimide reaction and (b) schematic representation of phototriggered protein release from the gel. (*Source*: Adapted with permission from Reference [98].)



4.18 Left: Chemical structure of a cross-linked pNIPAAm hydrogel functionalized with spiropyran and a schematic illustration of the photo-induced shrinking of the hydrogel. Right: Images of the hydrogel layer before (a) and after (b) the micropatterned light irradiation. Irradiation times were (●) 0 s, (◊) 1 s, and (□) 3 s. (c) Thickness change of the hydrogel layer in (●) nonirradiated and (○) irradiated region (3 s blue light irradiation) *vs* time. (*Source*: Adapted with permission from Reference [31].)

in effective cross-linking of the dextran and formation of the hydrogel. The green fluorescent protein (GFP) was encapsulated in this system. In the cross-linked system, GFP remains inside the gel but after UV light irradiation GFP can diffuse out of the gel and is released. This strategy works only with big molecules (such as protein, DNA, or drug with high molecular weight) which are not able to diffuse outside of the polymer network in the cross-linked form. Phototriggered shrinkage has also been achieved in a PNIPAM hydrogel modified with 1 mol% spirobenzopyran-modified acrylate.[31] An acidic aqueous solution of this polymer maintained in the dark forms a highly hydrated gel, since most of the spirobenzopyran is present in the positively charged open-ring form. Irradiation with blue light causes the transition to the closed and uncharged form of spirobenzopyran, leading to collapse of the hydrogel in the exposed area. Such shrinkage has been applied to generate a rewritable microrelief (Fig. 4.18).

Phototriggered delivery of Ca^{2+} cations has been used as a light-induced approach to cross-link alginate hydrogels.[99] Photolabile Ca^{2+} cages are chelators that change their affinity for Ca^{2+} upon light exposure from a K_d of several to hundreds of nM to several mM.[100] The affinity change is a consequence of a light-induced change in the molecular structure and can

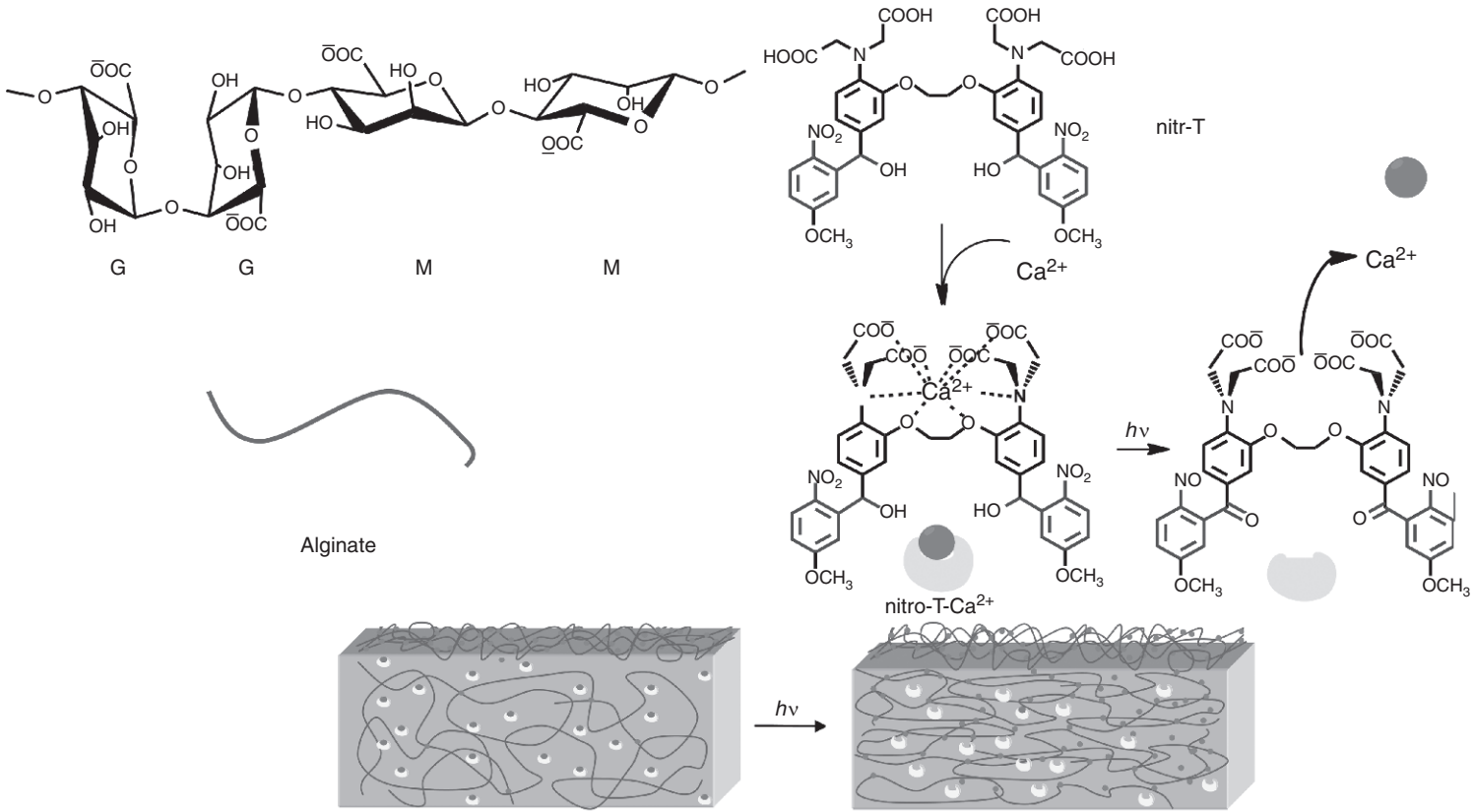
be used to change the local concentration of Ca^{2+}.[39,100] Cage compound nitr-T (Fig. 4.19) has been developed for this purpose and embedded in alginate solution.[101] Irradiation at 360 nm released Ca^{2+} cations which bond to adjacent α-L-guluronic acid (G) residues of alginate with chelating interaction of the carboxylic groups.[102] This interaction results in gelation and the resulting hydrogel displays a higher rheological modulus compared to the alginate hydrogel prepared by mixing $CaCl_2$ solution directly (Fig. 4.19).[103]

4.4.4 Photodegradable materials

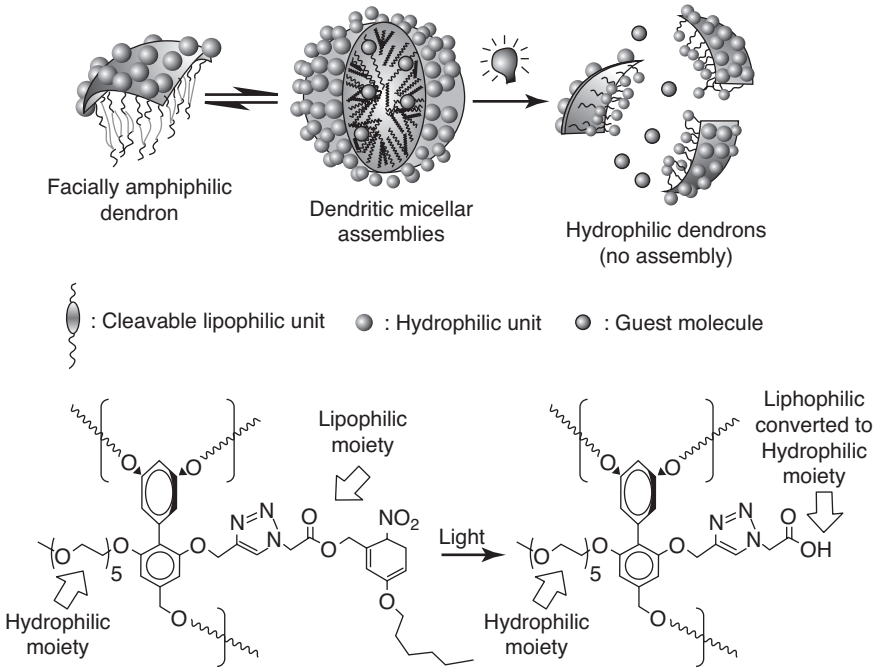
Polymers with photolabile groups in the main chain undergo chain breakage upon light illumination and can be classified as photodegradable materials. Most of the recent works use the *o*-nitrobenzyl chromophore, but a few other photodepolymerization strategies have also been applied. Silicon-containing polyureas undergo photodegradation upon irradiation at $\lambda >$ 300 nm due to the photo-induced single-electron transfer from the σ C–Si to the adjacent π C=O bond, followed by silyl group migration and solvolysis.[104] Biocompatible polyketals and polyacetals have been synthesized and were photolyzed by UV light at 248 nm into carbonyl and hydroxyl product through zwitterionic intermediates and were then applied for making cell patterns.[105] The photolysis requires low energy as a consequence of the ionic photo intermediate instead of a radical one. This property makes this system interesting for biomaterials applications, since the exposure conditions are compatible with living cells.

The diblock copolymer of PS and PEO with a photodegradable *o*-nitrobenzyl linker was applied to achieve ordered self-assembly nanostructures.[14,71] The copolymer self-assembled into highly ordered, hexagonally packed cylinders oriented perpendicular to the substrate with PS as the continuous phase. UV irradiation cleaves the two blocks by the photolysis reaction of *o*-nitrobenzyl. The free PEO block was washed with water which led to a nanoporous template.[14,38,71,106] Polymers with some kinds of metal–metal bonds (such as iron and molybdenum) in main chains of polyesters and polyamides lead to other kinds of photodegradable polymers which are sensitive to visible light. Films of such photodegrabable polymers are interesting for agriculture, since polymer film degradation can occur with daylight and the film does not need to be removed.[107]

Several applications of nitrobenzenyl derivatives have recently been reported. Figure 4.20 presents a photodegradable dendron with a hydrophilic and a lipophilic unit connected by the photocleavable group.[108] Micelles of this dendron have been used for encapsulating and light-mediated delivering of Nile red.



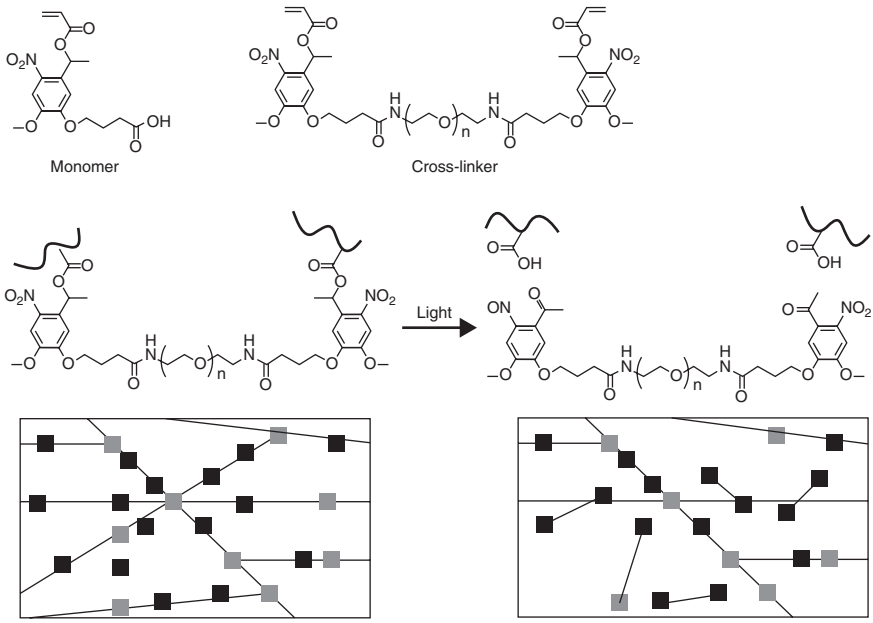
4.19 Model of phototriggering alginate hydrogel system with nitr-T-Ca^{2+} and its light-induced shrinking. (*Source*: Adapted with permission from Reference [103].)



4.20 Light-induced disassembly of dendritic micellar assemblies after light exposure. (*Source*: Adapted with permission from Reference [108].)

Photodegradable hydrogels containing poly(ethylene glycol) (PEG) chains cross-linked with photocleavable nitrobenzyl units (1 and 2 in Fig. 4.21) have been applied as 3D scaffolds for cell growth with light-tunable mechanical properties.[109] Using scanning lasers and two-photon excitation, micrometric resolution of the photodegradation process was possible. 3D channels with reduced cross-linking were created inside the hydrogel in order to direct cell migration.

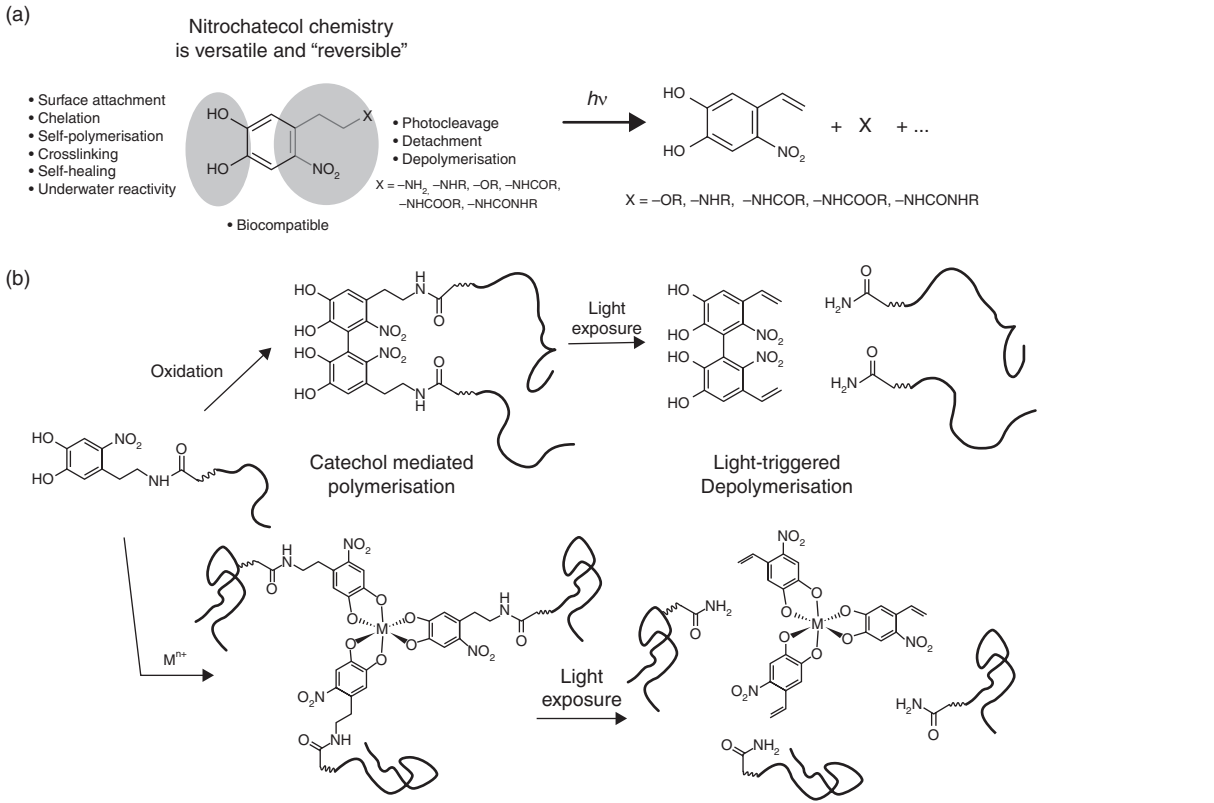
In a bioinspired approach, nitrodopamine has been used to end-cap a star PEG and form networks which are either covalently or metal cross-linked (Fig. 4.22). Upon UV irradiation, the nitrophenylethyl group photolysized and the hydrogel degraded. This bioinspired material retains the underwater bonding properties of the mussel (due to the catechol moieties), and incorporates the possibility of light-induced debonding. It represents a new generation of photodegradable biomaterials that can be widely used in biocompatible coating, culturing cell, and medical applications.[110]



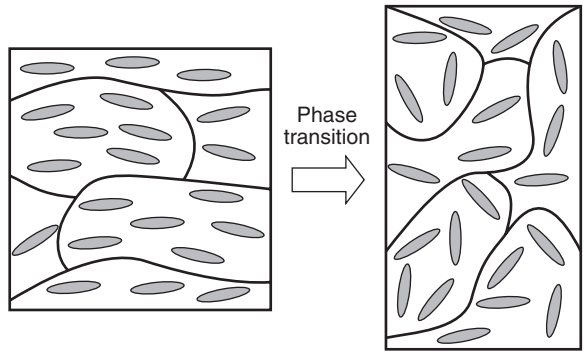
4.21 Photolabile acrylate monomer and bifunctional cross-linker; photodegradable hydrogels synthesized by free-radical polymerization of the PEG macromers, which are used as photolabile 3D substrates for dynamic cell culture. (*Source*: Adapted with permission from Reference [109a].)

4.4.5 Photoswitchable liquid crystalline elastomers (LCEs) for remote actuation

Photoswitchable units have been incorporated into liquid crystalline polymers. The chromophore is usually part of the mesogenic core and the light-induced molecular changes directly affect the degree of order of the mesophase and, consequently, properties such as Curie temperature,[111] molecular orientation, symmetry, transition temperatures, etc.[112] One issue of recent interest in photo-responsive LCEs is the possibility of generating photoswitchable actuators. Light exposure induces a change in the conformation of polymer chains from an extended to a coiled one and which results in a macroscopic shape change. This actuating principle has been applied for bending LCE films and micropillars, such as those shown in Fig. 4.23.[113] The first example of this kind was reported in 2003 with polymer **18**.[59] A 20 μm thin film of the LCE with the mesogens preferentially oriented in a direction parallel with the long axis was obtained. Film contraction in the



4.22 Structure of nitrodopamine derivatives and their photocleavage mechanisms. (a) Photolytic reaction of the *o*-nitrophenyl ethyl moiety. (b) Different strategies used to trigger bonding and debonding upon light exposure of nitrodopamine derivatives. (*Source*: Adapted with permission from Reference [110].)

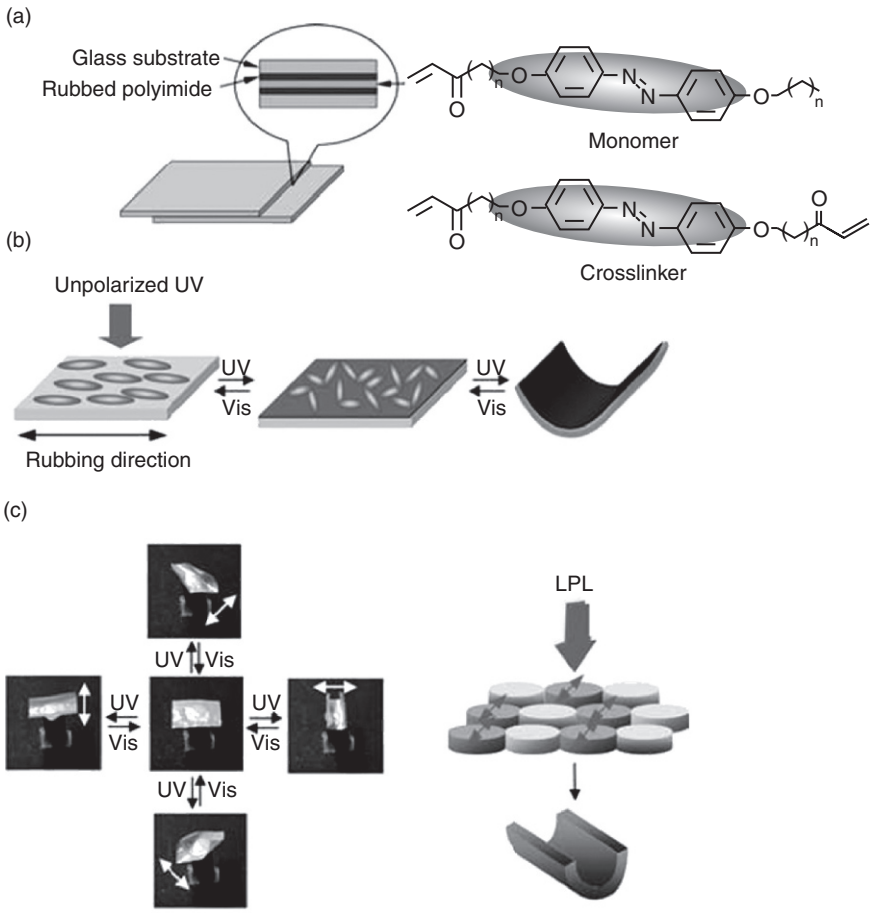


4.23 The deformation model of a LCE. In the LC phase, the anisotropic environment forces the polymer backbones to adopt extended chain conformation. During the phase transition to isotropic phase, the polymer regains its coiled conformation as a consequence of entropic driving, which gives rise to a macroscopic shape change. (*Source*: Adapted with permission from Reference [113a].)

direction of mesogenic units was observed upon exposure to UV light at 75°C as a consequence of *trans*-to-*cis* transition of azobenzyl, which induced nematic-isotropic phase transition.

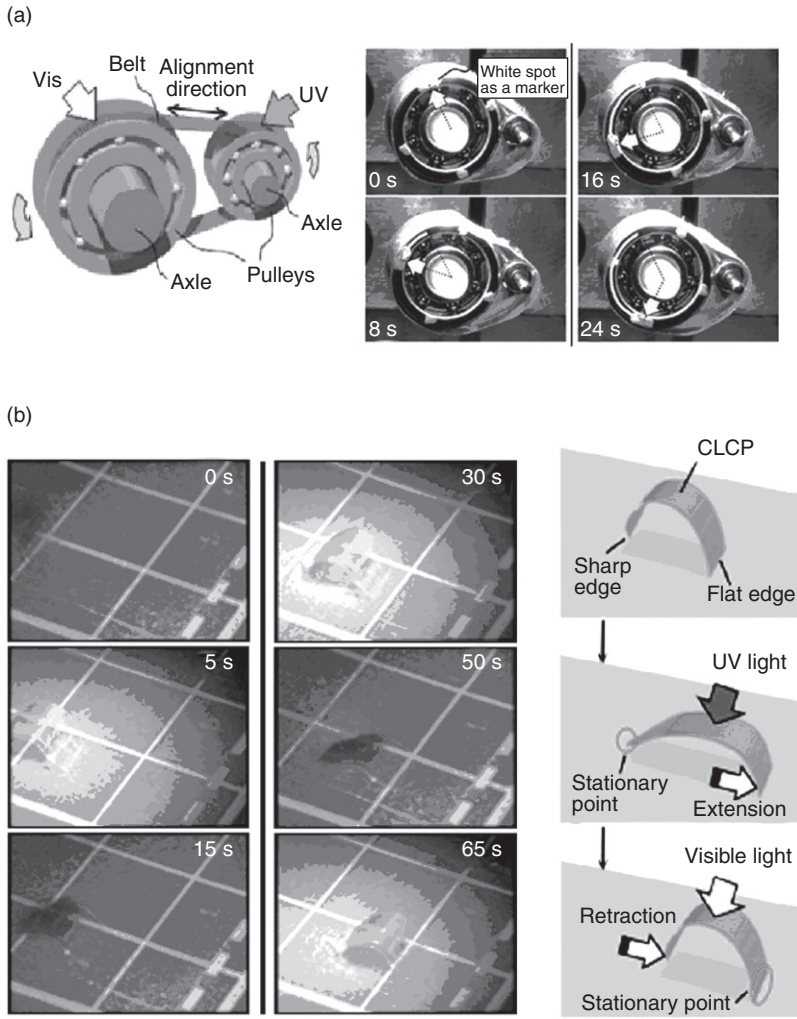
Figure 4.24 presents a different example where a freestanding LCE film was bent upon irradiation.[9] Monodomain LCE films with the mesogens aligned in parallel direction to the surfaces were generated by the method shown in Fig. 4.24a.[15a] *In situ* polymerization and cross-linking of the film was carried out in the liquid crystal (LC) phase. In the resulting self-supporting LCE films, the network structure retained the orientation of the mesogens. UV irradiation caused macroscopic bending of the film as a consequence of the contraction induced by the transition of the LC phase into the isotropic phase. Such deformation was reversible by irradiating with alternating UV and visible light sources (Fig. 4.24b). When polarized light was used, direction-controllable bending was achieved in a polydomain LCE film, as shown in Fig. 4.24c.[114] Recently, similar effects were observed with fibers which can bend to any bend towards a directional light source, like a 'sunflower'.[115]

The contraction and bending of LCEs have inspired interesting application attempts (Fig. 4.25). An azobenzenyl-containing LCE layer was attached to a flexible polyethylene sheet and used as a photo-driven belt (Fig. 4.25a).[116] Both UV and visible light were shone simultaneously from different directions and a rotation of the belt was induced. This rotation was used to drive a motor device in a counterclockwise



4.24 Photo-responsive freestanding LCE film. (a) Scheme of the preparation process of freestanding LCE film using an azobenzyl-based LC monomer and a cross-linker. (b) Phototriggered bending mechanism of the monodomain LCE films. (c) Control the bending direction of LCE films by linearly polarized light and its plausible mechanism.[15a] (*Source*: Adapted with permission from reference [15a].)

direction at room temperature. It represents the first realization of light-driven plastic motors in which light energy was directly converted into mechanically rotational energy. Exploiting bending and stretching effects of a LCE film, bioinspired propulsion was further demonstrated (Fig. 4.25b). The movement was driven by cyclic photo-induced bending and extension.[117,118]



4.25 Photo-induced sophisticated 3D motions of a LCE film laminated on a flexible polyethylene sheet. (a) Schematic illustration of a light-driven plastic motor and photographs of time profiles of the rotation. (*Source*: Adapted with permission from Reference [116].) (b) Photographs of photo-induced inchworm walk and the plausible mechanism. (*Source*: Adapted with permission from reference [117].)

4.5 Conclusions and future trends

The future of photo-responsive polymers and smart materials derived from them will depend on the research development in different directions. The development of photo-responsive units acts as the engine of photo-

responsive polymer systems. New and more efficient photosensitive molecular units and switching strategies are required, as well as chromophores sensitive to long wavelengths for compatibility with living organisms and tissues and applications in the biomedical area. The development of polymerization strategies able to incorporate the chromophores at selected positions in complex macromolecular architectures is also an important area, that is, by mature living control radical polymerization techniques.[119] Controlled drug delivery will remain one of the main fields in the application of photosensitive polymers. Although many systems have been tested *in vitro* with a dye or a drug, *in vivo* applications are scarce and require further development. Close cooperation between organic chemists, polymer chemists, biologists, and medical doctors will be required to push this research to the interdisciplinary level.[120] Light-triggered actuators will also be an area for future research. Until now, these have been based on azobenzyl-containing LCEs. However, indirect strategies can also achieve similar effects and may also extend the irradiation wavelength to broader regions.[121] All of these areas will certainly grow further in the future and expand the application of these systems to unforeseen fields.

4.6 References

1. (a) M. Irie and T. Ikeda (1997) in *Photoresponsive polymers*, Vol. Dekker, pp. 65–116; (b) V. Shibaev, A. Bobrovsky and N. Boiko (2003), *Prog. Polym. Sci.*, **28**, 729–836; (c) Y. Zhao (2007) *Chem. Rec.*, **7**, 286–294; (d) U. Krauss, J. Lee, S. J. Benkovic and K.-E. Jaeger (2010), *Microb Biotechnol*, **3**, 15–23.
2. (a) S. Shinkai, H. Kinda and O. Manabe (1982), *J. Am. Chem. Soc.*, **104**, 2933–2934; (b) I. Nor, R. Enea, V. Hurduc and M. Bercea (2007), *J. Optoelectron. Adv. Mater.*, **9**, 3639–3644; (c) C.-T. Lai and J.-L. Hong (2010), *J. Phys. Chem. B*, **114**, 10302–10310; (d) S. Gupta, P. Uhlmann, M. Agrawal, S. Chapuis, U. Oertel and M. Stamm (2008), *Macromolecules*, **41**, 2874–2879; (e) J. Ruchmann, S. C. Sebai and C. Tribet (2011), *Macromolecules*, **44**, 604–611; (f) G. J. Everlof and G. D. Jaycox (2000), *Polymer*, **41**, 6527–6536; (g) M. Irie (1993) in *Photo- and Chemical-Responsive Polymer Solution and Gel Systems*, *Vol. B* Elsevier, pp. 159–164.
3. (a) A. A. Zakhidov and K. Yoshino (1995), *Synth. Met.*, **71**, 1875–1876; (b) Y. Wu, Q. Zhang, A. Kanazawa, T. Shiono, T. Ikeda and Y. Nagase (1999), *Macromolecules*, **32**, 3951–3956; (c) R. Hidayat, Y. Nishihara, A. Fujii, M. Ozaki, K. Yoshino and E. Frankevich (2002), *Phys. Rev. B: Condens. Matter Mater. Phys.*, **66**, 075214/075211–075214/075217; (d) S. Sajti, A. Kerekes, P. S. Ramanujam and E. Lorincz (2002), *Appl. Phys. B: Lasers Opt.*, **75**, 677–685; (e) S. Pandey, B. Kolli, S. P. Mishra and A. B. Samui (2012) *J. Polym. Sci., Part A: Polym. Chem.*, **50**, 1205–1215; (f) Y. Wang, S. Lin, M. Zang, Y. Xing, X. He, J. Lin and T. Chen (2012), *Soft Matter*, **8**, 3131–3138.
4. (a) C. Chen, G. Liu, X. Liu, S. Pang, C. Zhu, L. Lv and J. Ji (2011), *Polym. Chem.*, **2**, 1389–1397; (b) J. He and Y. Zhao (2011), *Dyes Pigm.*, **89**, 278–283; (c) C.-J. Chen, G.-Y. Liu, X.-S. Liu, D.-D. Li and J. Ji (2012), *New J. Chem.*, **36**, 694–701; (d) D. Han, X. Tong and Y. Zhao (2012), *Langmuir*, **28**, 2327–2331.

5. (a) S. F. B. De, J. D. T. Guerreiro, M. Ma, D. G. Anderson, C. L. Drum, R. D. Sinisterra and R. Langer (2010), *J. Mater. Chem.*, **20**, 9910–9917; (b) K. H. Fries, J. D. Driskell, S. Samanta and J. Locklin (2010), *Anal. Chem.*, **82**, 3306–3314; (c) R. Byrne, C. Ventura, L. F. Benito, A. Walther, A. Heise and D. Diamond (2011), *Biosens. Bioelectron.*, **26**, 1392–1398.
6. (a) S. Mayer and R. Zentel (1998), *Macromol. Chem. and Phys.*, **199**, 1675–1682; (b) W. Zhang, K. Yoshida, M. Fujiki and X. Zhu (2011), *Macromolecules*, **44**, 5105–5111.
7. (a) E. Kim, S. Y. Cho and K.-H. Ahn (2005), *Mol. Cryst. Liq. Cryst.*, **430**, 135–145; (b) K. Uchida, A. Takata, S.-i. Ryo, M. Saito, M. Murakami, Y. Ishibashi, H. Miyasaka and M. Irie (2005), *J. Mater. Chem.*, **15**, 2128–2133.
8. B. L. Feringa, R. A. van Delden, N. Koumura and E. M. (2000) Geertsema, *Chem. Rev.*, **100**, 1789–1816.
9. T. Ikeda, J.-i. Mamiya and Y. Yu (2007), *Angew. Chem., Int. Ed.*, **46**, 506–528.
10. Y. Zhao (2009), *J. Mater. Chem.*, **19**, 4887–4895.
11. G. Pasparakis, T. Manouras, P. Argitis and M. Vamvakaki (2012), *Macromol. Rapid Commun.*, **33**, 183–198.
12. J.-M. Schumers, C.-A. Fustin and J.-F. Gohy (2010), *Macromol. Rapid Commun.*, **31**, 1588–1607.
13. Y. Zhao (2012), *Macromolecules*, **45**, 3647–3657.
14. H. Zhao, E. S. Sterner, E. B. Coughlin and P. Theato (2012), *Macromolecules*, **45**, 1723–1736.
15. (a) H. Yu and T. Ikeda (2011), *Adv. Mater.*, **23**, 2149–2180; (b) T. Ikeda, J. I. Mamiya and Y. Yu, *Angew. Chem. Int. Ed.* 2007, **46**, 506–528.
16. H. Wondraczek, A. Kotiaho, P. Fardim and T. Heinze (2011), *Carbohydr. Polym.*, **83**, 1048–1061.
17. S. Al-Malaika, C. Hewitt and H. H. Sheena (2010) in *Photodegradable Polymers*, *Vol.* John Wiley & Sons, Inc., pp. 603–626.
18. F. Ercole, T. P. Davis and R. A. Evans (2010), *Polym. Chem.*, **1**, 37–54.
19. J. Cui, V. San Miguel and A. del Campo (2013), *Macromol. Rapid Commun.*, DOI: 10.1002/marc.201200634.
20. A. Suzuki and T. Tanaka, *Nature* 1990, **346**, 345–347.
21. (a) Y. Wang, N. Ma, Z. Wang and X. Zhang (2007), *Angew. Chem. Int. Ed.*, **119**, 2881–2884; (b) I. Willerich and F. Gröhn (2010), *Angew. Chem., Int. Ed.*, **49**, 8104–8108.
22. S. Yagai and A. Kitamura (2008), *Chem. Soc. Rev.*, **37**, 1520–1529.
23. C. J. Barrett, J.-I. Mamiya, K. G. Yager and T. Ikeda (2007), *Soft Matter*, **3**, 1249–1261.
24. G. Maxein and R. Zentel (1995), *Macromolecules*, **28**, 8438–8440.
25. (a) A. Kozanecka-Szmigiel, D. Szmigiel, K. Switkowski, E. Schab-Balcerzak and M. Siwy (2011), *Opt. Appl.*, **41**, 777–785; (b) A. Kravchenko, A. Shevchenko, V. Ovchinnikov, A. Priimagi and M. Kaivola (2011), *Adv. Mater.*, **23**, 4174–4177; (c) R. Alicante, R. Cases, B. Villacampa and E. Blasco (2012), *Macromol. Chem. Phys.*, **213**, 776–783; (d) E. Schab-Balcerzak, M. Grucela-Zajac, A. Kozanecka-Szmigiel and K. Switkowski (2012), *Opt. Mater.*, **34**, 733–740.
26. (a) R. Barille, S. Ahmadi-Kandjani, E. Ortyl, S. Kucharski and J.-M. Nunzi (2009), *New J. Chem.*, **33**, 1207–1210; (b) J. Royes, J. Rebole, L. Custardoy, N. Gimeno, L. Oriol, R. M. Tejedor and M. Pinol (2012), *J. Polym. Sci., Part A: Polym. Chem.*, **50**, 1579–1590.

27. S. V. Paramonov, V. Lokshin and O. A. Fedorova (2011), *J. Photochem. Photobiol., C*, **12**, 209–236.
28. S. H. Anastasiadis, M. I. Lygeraki, A. Athanassiou, M. Farsari and D. Pisignano (2008), *J. Adhes. Sci. Technol.*, **22**, 1853–1868.
29. H.-i. Lee, W. Wu, J. K. Oh, L. Mueller, G. Sherwood, L. Peteanu, T. Kowalewski and K. Matyjaszewski (2007), *Angew. Chem. Int. Ed.*, **46**, 2453–2457.
30. J. Andersson, S. Li, P. Lincoln and J. Andréasson (2008), *J. Am. Chem. Soc.*, **130**, 11836–11837.
31. A. Szilagyi, K. Sumaru, S. Sugiura, T. Takagi, T. Shinbo, M. Zrinyi and T. Kanamori (2007), *Chem. Mater.*, **19**, 2730–2732.
32. A. Nayak, H. Liu and G. Belfort (2006), *Angew. Chem. Int. Ed.*, **45**, 4094–4098.
33. M. Walko and B. L. Feringa (2007), *Chem. Commun*, 1745–1747.
34. Q. Luo, H. Cheng and H. Tian (2011), *Polym. Chem.*, **2**, 2435–2443.
35. C. Cardenas-Daw, A. Kroeger, W. Schaertl, P. Froimowicz and K. Landfester (2012), *Macromol. Chem. and Phys.*, **213**, 144–156.
36. (a) S. Arumugam and V. V. Popik (2009), *J. Am. Chem. Soc.*, **131**, 11892–11899; (b) S. Arumugam and V. V. Popik (2011), *J. Am. Chem. Soc.*, **133**, 5573–5579; (c) S. Arumugam and V. V. Popik (2011), *J. Am. Chem. Soc.*, **133**, 15730–15736; (d) S. Arumugam, S. V. Orski, J. Locklin and V. V. Popik (2012), *J. Am. Chem. Soc.*, **134**, 179–182.
37. (a) A. A. Brown, O. Azzaroni and W. T. S. Huck (2009), *Langmuir*, **25**, 1744–1749; (b) J. Cui, O. Azzaroni and A. del Campo (2011), *Macromol. Rapid Commun.*, **32**, 1699–1703; (c) A. A. Brown, O. Azzaroni, L. M. Fidalgo and W. T. S. Huck (2009), *Soft Matter*, **5**, 2738–2745.
38. P. Theato (2011), *Angew. Chem., Int. Ed.*, **50**, 5804–5806.
39. G. Mayer and A. Heckel (2006), *Angew. Chem. Int. Ed.*, **45**, 4900–4921.
40. M. Goeldner and R. Givens (2005), *Dynamic Studies in Biology: Phototriggers, Photoswitches and Caged Biomolecules*, Wiley VCH, Weinheim, p. 159.
41. F. Stellacci, C. Bertarelli, F. Toscano, M. C.Gallazzi, G. Zotti and G. Zerbi (1999), *Adv. Mater.*, **11**, 292–295.
42. J. Areephong, T. Kudernac, J. J. D. de Jong, G. T. Carroll, D. Pantorott, J. Hjelm, W. R. Browne and B. L. Feringa (2008), *J. Am. Chem. Soc.*, **130**, 12850–12851.
43. C. Bertarelli, A. Bianco, V. Boffa, M. Mirenda, M. C. Gallazzi and G. Zerbi (2004), *Adv. Funct. Mater.*, **14**, 1129–1133.
44. T. Kawai, T. Kunitake and M. Irie (1999), *Chem. Lett.*, **9**, 905–906.
45. E. Kim and H. W. Lee (2006), *J. Mater. Chem.*, **16**, 1384–1389.
46. (a) H. Choi, H. Lee, Y. Kang, E. Kim, S. O. Kang and J. Ko (2005), *J. Org. Chem.*, **70**, 8291–8297; (b) T. Kawai, Y. Nakashima and M. Irie (2005), *Adv. Mater.* **17**, 309–314.
47. M. J. Marsella, Z.-Q. Wang and R. H. Mitchell (2000), *Org. Lett.*, **2**, 2979–2982.
48. J. Yang and M.-K. Ng (2006), *Synthesis*, **18**, 3075–3079.
49. P. J. Roth, F. D. Jochum, F. R. Forst, R. Zentel and P. Theato (2010), *Macromolecules*, **43**, 4638–4645.
50. A. P. Goodwin, J. L. Mynar, Y. Ma, G. R. Fleming and J. M. J. Frechet (2005), *J. Am. Chem. Soc.*, **127**, 9952–9953.
51. H. Akiyama and N. Tamaoki (2007), *Macromolecules*, **40**, 5129–5132.
52. F. D. Jochum, B. L. zur, P. J. Roth and P. Theato (2009), *Macromolecules*, **42**, 7854–7862.

53. D. Pijper, M. G. M. Jongejan, A. Meetsma and B. L. Feringa (2008), *J. Am. Chem. Soc*, **130**, 4541–4552.
54. M. M. Green, J.-W. Park, T. Sato, A. Teramoto, S. Lifson, R. L. B. Selinger and J. V. Selinger (1999), *Angew. Chem. Int. Ed.*, **38**, 3138–3154.
55. Y. Zhao, L. Tremblay and Y. Zhao (2011), *Macromolecules*, **44**, 4007–4011.
56. Y. Zhao, L. Tremblay and Y. Zhao (2010), *J. Polym. Sci., Part A: Polym. Chem.*, **48**, 4055–4066.
57. I. Tomatsu, A. Hashidzume and A. Harada (2006), *J. Am. Chem. Soc.*, **128**, 2226–2227.
58. (a) I. Tomatsu, A. Hashidzume and A. Harada, (2005) *Macromolecules*, **38**, 5223–5227; (b) X. Liao, G. Chen, X. Liu, W. Chen, F. Chen and M. Jiang (2010), *Angew. Chem. Int. Ed.*, **49**, 4409–4413.
59. M. H. Li, P. Keller, B. Li, X. Wang and M. Brunet (2003), *Adv. Mater.*, **15**, 569–572.
60. R. Byrne, C. Ventura, L. F. Benito, A. Walther, A. Heise and D. Diamond (2011), *Biosens. Bioelectron.*, **26**, 1392–1398.
61. J. Cui, N. T. Huong, M. Ceolín, R. d. Berger, O. Azzaroni and A. del Campo, (2012) *Macromolecules*, **45**, 3213–3220.
62. A. Brunsen, J. Cui, M. Ceolin, A. del, G. J. A. A. Soler-Illia and O. Azzaroni (2012), *Chem. Commun.*, **48**, 1422–1424.
63. E. J. Foster, E. B. Berda and E. W. Meijer (2009), *J. Am. Chem. Soc.*, **131**, 6964–6966.
64. (a) T. F. A. De Greef, M. M. J. Smulders, M. Wolffs, A. P. H. J. Schenning, R. P. Sijbesma and E. W. Meijer (2009), *Chem. Rev.*, **109**, 5687–5754; (b) R. P. Sijbesma, F. H. Beijer, L. Brunsveld, B. J. B. Folmer, J. H. K. K. Hirschberg, R. F. M. Lange, J. K. L. Lowe and E. W. Meijer (1997), *Science*, **278**, 1601–1604.
65. M. T. Frey and Y.-l. Wang (2009), *Soft Matter*, **5**, 1918–1924.
66. X. Tong, G. Wang, A. Soldera and Y. Zhao (2005), *J. Phys, Chem. B*, **109**, 20281–20287.
67. (a) J. Jiang, B. Qi, M. Lepage and Y. Zhao (2007), *Macromolecules*, **40**, 790–792; (b) J. Babin, M. lepage and Y. Zhao (2008), *Macromolecules*, **41**, 126–1253.
68. H. Yu, T. Iyoda and T. Ikeda (2006), *J. Am. Chem. Soc.*, **128**, 11010–11011.
69. J. Jiang, X. Tong and Y. Zhao (2005), *J. Am. Chem. Soc.*, **127**, 8290–8291.
70. (a) J. Babin, M. Pelletier, M. Lepage, J.-F. Allard, D. Morris and Y. Zhao (2009), *Angew. Chem. Int. Ed.*, **48**, 3329–3332; (b) J. Jiang, X.Tong, D. Morris and Y. Zhao (2006), *Macromolecules*, **39**, 4633–4640; (c) J.-M. Schumers, O. Bertrand, C.-A. Fustin and J.-F. Gohy (2012), *J. Polym. Sci., Part A: Polym. Chem.*, **50**, 599–608.
71. M. Kang and B. Moon (2009), *Macromolecules*, **42**, 455–458.
72. J.-M. Schumers, J.-F. Gohy and C.-A. Fustin (2010), *Polym. Chem.*, **1**, 161–163.
73. D. L. Jiang and T. Aida (1997), *Nature*, **388**, 454–456.
74. D. S. Bradshaw and D. L. Andrews (2011), *Polymers*, **3**, 2053–2077.
75. R. Deloncle and A.-M. Caminade (2010), *J. Photochem. Photobiol. C*, **11**, 25–45.
76. X. Zhang, C. Wang, X. Lu and Y. Zeng (2011), *J. Appl. Poly. Sci.*, **120**, 3065–3070.
77. T.-T.-T. Nguyen, D. Turp, D. Wang, B. Nolscher, F. Laquai and K. Mullen (2011), *J. Am. Chem. Soc.*, **133**, 11194–11204.

78. T. Koyama, K. Hatano, K. Matsuoka, Y. Esumi and D. Terunuma (2009), *Molecules*, **14**, 2226–2234.
79. J. Hayakawa, A. Momotake and T. Arai (2003), *Chem. Commun.*, 94–95.
80. A. R. Cano-Marín, E. Díez-Barra and J. Rodríguez-López (2005), *Tetrahedron*, **61**, 395–400.
81. B. Yu, X. Jiang, R. Wang and J. Yin (2010), *Macromolecules*, **43**, 10457–10465.
82. (a) R. M. Kevwitch and D. V. McGrath (2007), *New J. Chem.*, **31**, 1332–1336; (b) R. Kevwitch and D. V. McGrath (2001), *Polym. Mater. Sci. Eng.*, **84**, 749–750; (c) R. M. Kevwitch and D. V. McGrath (2002), *Synthesis*, 1171–1176.
83. S. Watanabe, M. Sato, S. Sakamoto, K. Yamaguchi and M. Iwamura (2000), *J. Am. Chem. Soc.*, **122**, 12588–12589.
84. M. Smet, L.-X. Liao, W. Dehaen and D. V. McGrath (2000), *Org. Lett.*, **2**, 511–513.
85. Y. Li, X. Jia, M. Gao, H. He, G. Kuang and Y. Wei (2010), *J. Polym. Sci. Part A: Polym. Chem.*, **48**, 551–557.
86. B. J. B. Folmer, E. Cavini, R. P. Sijbesma and E. W. Meijer (1998), *Chem. Commun.*, 1847–1848.
87. (a) E. B. Berda, E. J. Foster and E. W. Meijer (2010), *Macromolecules*, **43**, 1430–1437; (b) J. Cui and A. del Campo (2012), *Chem. Commun.*, **48**, 9302–9304; (c) J. Cui, D. Wang, K. Koynov, A. del Campo, *Submitted*. 2nd Revision under review.
88. P. Kuad, A. Miyawaki, Y. Takashima, H. Yamaguchi and A. Harada (2007), *J. Am. Chem. Soc.*, **129**, 12630–12631.
89. R. Dong, Y. Liu, Y. Zhou, D. Yan and X. Zhu (2011), *Polym. Chem.*, **2**, 2771–2774.
90. B. Yan, J.-C. Boyer, N. R. Branda and Y. Zhao (2011), *J. Am. Chem. Soc.*, **133**, 19714–19717.
91. (a) R. Barille, R. Janik, S. Kucharski, J. Eyer and F. Letournel (2011), *Colloids Surf., B*, **88**, 63–71; (b) S. Matsumoto, S. Yamaguchi, S. Ueno, H. Komatsu, M. Ikeda, K. Ishizuka, Y. Iko, K. V. Tabata, H. Aoki, S. Ito, H. Noji and I. Hamachi (2008), *Chem. Eur. J.*, **14**, 3977–3986; (c) J. Kim, J. A. Hanna, M. Byun, C. D. Santangelo and R. C. Hayward (2012), *Science*, **335**, 1201–1205.
92. (a) Y.-H. Gong, C. Li, J. Yang, H.-Y. Wang, R.-X. Zhuo and X.-Z. Zhang (2011), *Macromolecules*, **44**, 7499–7502; (b) N. Lomadze, A. Kopyshev, J. r. Rühe and S. Santer (2011), *Macromolecules*, **44**, 7372–7377; (c) X. Wang, J. Yin and X. Wang (2011), *Macromolecules*, **44**, 6856–6867.
93. (a) I. Tokarev and S. Minko (2009), *Soft Matter*, **5**, 511–524; (b) S. Chaterji, I. K. Kwon and K. Park (2007), *Prog. Polym. Sci.*, **32**, 1083–1122; (c) I. Tokarev and S. Minko (2010), *Adv. Mater.*, **22**, 3446–3462; (d) T. Miyata (2010), *Polym. J.*, **42**, 277–289; (e) V. Kozlovskaya, E. Kharlampieva, I. Erel and S. A. Sukhishvili (2009), *Soft Matter*, **5**, 4077–4087; (f) D. Kuckling (2009), *Colloid Polym. Sci.*, **287**, 881–891; (g) M. Prabaharan and J. F. Mano (2006), *Macromol. Biosci.*, **6**, 991–1008; (h) J. Wang, Z. Chen, M. Mauk, K.-S. Hong, M. Li, S. Yang and H. H. Bau (2005), *Biomed. Microdevices*, **7**, 313–322; (i) L. A. Lyon, Z. Meng, N. Singh, D. Sorrell Courtney and A. St John (2009), *Chem. Soc. Rev.*, **38**, 865–874.
94. R. Lovrien (1967), *Proc. Natl. Acad. Sci. U.S.A.*, **57**, 236–242.
95. G. Van Der Veen and W. Prins (1971), *Nature*, **230**, 70–72

96. M. Irie and D. Kungwatchakun (1984), *Makromol. Chem., Rapid Commun.*, **5**, 829–832.
97. (a) M. Irie and D. Kunwatchakun (1986), *Macromolecules*, **19**, 2476–2480; (b) A. Mamada, T. Tanaka, D. Kungwatchakun and M. Irie (1990), *Macromolecules*, **23**, 1517–1519.
98. K. Peng, I. Tomatsu and A. Kros (2010), *Chem. Commun.*, **46**, 4094–4096.
99. (a) A. D. Augst, H. J. Kong and D. J. Mooney (2006), *Macromol. Biosci.*, **6**, 623–633; (b) H. Park and K. Y. Lee (2008), *Nat.-Polym. Biomed. Appl.*, 515–532.
100. G. C. R. Ellis-Davies (2008), *Chem. Rev.*, **108**, 1603–1613.
101. J. Cui, R. A. Gropeanu, D. R. Stevens, J. Rettig and A. del Campo (2012), *J. Am. Chem. Soc.*, **134**, 7733–7740.
102. B. T. Stokke, K. I. Draget, O. Smidsrod, Y. Yuguchi, H. Urakawa and K. Kajiwara (2000), *Macromolecules*, **33**, 1853–1863.
103. J. Cui, M. Wang, Y. Zheng, G. Rodríguez Muñiz and A. del Campo, *Submitted*.
104. J. R. Hwu and K. Y. King (2005), *Chem. Eur. J.*, **11**, 3805–3815.
105. G. Pasparakis, T. Manouras, A. Selimis, M. Vamvakaki and P. Argitis (2011), *Angew. Chem., Int. Ed.*, **50**, 4142–4145.
106. H. Zhao, W. Gu, E. Sterner, T. P. Russell, E. B. Coughlin and P. Theato (2011), *Macromolecules*, **44**, 6433–6440.
107. D. R. Tyler (2003), *Coord. Chem. Rev.*, **246**, 291–303.
108. V. Yesilyurt, R. Ramireddy and S. Thayumanavan (2011), *Angew. Chem., Int. Ed.*, 50, 3038–3042.
109. (a) A. M. Kloxin, A. M. Kasko, C. N. Salinas and K. S. Anseth (2009), *Science*, **324**, 59–63; (b) A. M. Kloxin, J. A. Benton and K. S. Anseth (2009), *Biomaterials*, **31**, 1–8; (c) A. M. Kloxin, M. W. Tibbitt, A. M. Kasko, J. A. Fairbairn and K. S. Anseth (2010), *Adv. Mater.*, **22**, 61–66.
110. Z. Shafiq, J. Cui, L. Pastor-Pérez, V. S. Miguel, R. A. Gropeanu, C. Serrano and A. del Campo (2012), *Angew. Chem. Int. Ed.*, **124**, 4408–4411.
111. P. Beyer, M. Krueger, F. Giesselmann and R. Zentel (2007), *Adv. Funct. Mater.*, **17**, 109–114.
112. T. Seki (2007), *Bull. Chem. Soc. Jpn.*, **80**, 2084–2109.
113. (a) C. Ohm, M. Brehmer and R. Zentel (2010), *Adv. Mater.*, **22**, 3366–3387; (b) Q. Qian, J. Chen, M.-H. Li, P. Keller and D. He (2012), *J. Mater. Chem.*, **22**, 4669–4674.
114. Y. Yu, M. Nakano and T. Ikeda (2003), *Nature*, **425**, 145.
115. T. Yoshino, M. Kondo, J.-I. Mamiya, M. Kinoshita, Y. Yu and T. Ikeda (2010), *Adv. Mater.*, **22**, 1361–1363.
116. M. Yamada, M. Kondo, J.-I. Mamiya, Y. Yu, M. Kinoshita, C. J. Barrett and T. Ikeda (2008), *Angew. Chem. Int. Ed.*, **47**, 4986–4988.
117. M. Yamada, M. Kondo, R. Miyasato, Y. Naka, J.-i. Mamiya, M. Kinoshita, A. Shishido, Y. Yu, C. J. Barrett and T. Ikeda (2009), *J. Mater. Chem.*, **19**, 60–62.
118. M. Camacho-Lopez, H. Finkelmann, P. Palffy-Muhoray and M. Shelley (2004), *Nat. Mater.*, **3**, 307–310.
119. (a) C. J. Hawker, A. W. Bosman and E. Harth (2001), *Chem. Rev.*, **101**, 3661–3688; (b) M. Kamigaito, T. Ando and M. Sawamoto (2001), *Chem. Rev.*, **101**, 3689–3746; (c) K. Matyjaszewski and J. Xia (2001), *Chem. Rev.*, **101**, 2921–2990.

120. (a) G. Cosa, M. Lukeman and J. C. Scaiano (2009), *Acc. Chem. Res.*, **42**, 599–607; (b) S. Mizukami, M. Hosoda, T. Satake, S. Okada, Y. Hori, T. Furuta and K. Kikuchi (2010), *J. Am. Chem. Soc.*, **132**, 9524–9525; (c) H.-C. Kim, S. Hartner, M. Behe, T. M. Behr and N. A. Hampp (2006), *J. Biomed. Opt.*, **11**, 034024/034021–034024/034029; (d) F. Tanaka, T. Mochizuki, X. Liang, H. Asanuma, S. Tanaka, K. Suzuki, S.-i. Kitamura, A. Nishikawa, K. Ui-Tei and M. Hagiya (2010), *Nano Lett.*, **10**, 3560–3565; (e) N. K. Mal, M. Fujiwara and Y. Tanaka (2003), *Nature*, **421**, 350–353.
121. N. Torras, K. E. Zinoviev, J. E. Marshall, E. M. Terentjev and J. Esteve (2011), *Appl. Phys. Lett.*, **99**, 254102/254101–254102/254103.

5

Magnetically responsive polymer gels and elastomers: properties, synthesis and applications

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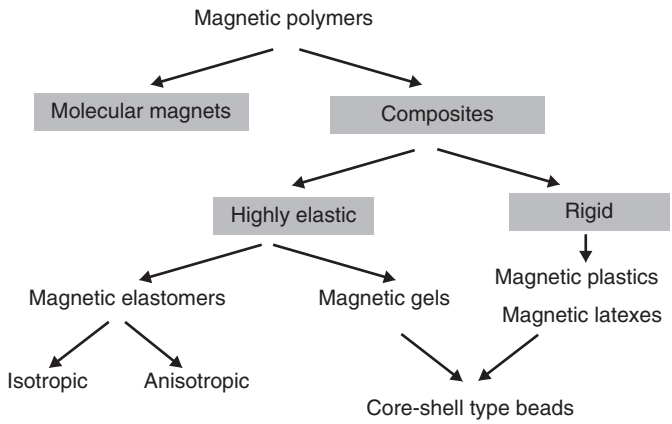
Abstract: The new generation of magnetic gels and elastomers represents a new type of composite, consisting of small (mainly nano-sized) magnetic particles dispersed in a high elastic polymeric matrix. The particles couple the shape of the elastomer to the external magnetic fields. Since the particles cannot leave the polymer matrix, all of the forces acting on the particles are transmitted directly to the polymer chains resulting in either locomotion or deformation. Shape distortion occurs instantaneously and disappears abruptly when external fields are applied or removed, respectively. The combination of magnetic and elastic properties leads to a number of striking phenomena that are exhibited in response to impressed magnetic fields. Giant deformational effect, tuneable elastic modulus, non-homogeneous deformation and quick response to magnetic field open new opportunities for using such materials for various applications.

Key words: magnetic gels, magneto-elasticity, non-uniform deformation, magnetite, magnetic nanoparticles, swelling, stress–strain, elastic modulus.

5.1 Introduction

Molecular magnets are systems where permanent magnetization and magnetic hysteresis can be achieved as a purely one-molecule phenomenon. These molecular magnets belong to a field that is still at an early stage of development. Their magnetic properties appear at extremely low temperatures and the magnetic response is rather weak (Miller and Eptein, 1998; Sato *et al.*, 1996; Verdaguer, 1996). Another possibility for the development of magnetic polymers is to apply flexible polymer composites containing magnetic particles. The variety of magnetic polymers is shown in Fig. 5.1.

Composite materials consisting of rather rigid polymeric matrices filled with magnetic particles have been known for a long time and are called magnetic plastics. These materials are successfully used as permanent



5.1 Family of magnetic polymers.

magnets, magnetic cores and connecting and fixing elements in many areas. These traditional magnetic elastomers have low flexibility and, practically, do not change their size, shape or elastic properties in the presence of external magnetic field.

The new generation of magnetic gels and elastomers represents a new type of composite, consisting of small (mainly nano-sized) magnetic particles dispersed in a high elastic polymeric matrix. Several terms like magnetic elastomers, magnetoactive polymers, magnetoelasts (Bossis *et al.*, 2004; Carlson and Jolly, 2000; Ginder and Davis, 1994; Ginder *et al.*, 1999, 2002; Jolly *et al.*, 1996; Shiga *et al.*, 1993, 1995) as well as magnetic gels, ferrogels, magnetorheological gels (Barsi and Zrinyi, 1998; Barsi *et al.*, 1996; Filipcsei *et al.*, 2007; Mitsumata *et al.*, 1999; Szabo *et al.*, 1997, 1998, 2000; Török *et al.*, 2000; Zrinyi, 1997; Zrinyi *et al.*, 1996, 1997a, 1997b, 1998, 1999a, 1999b, 1999c, 2000, 2002) are used to refer to magnetic soft materials. These materials are a specific subset of smart materials, which can adaptively change their physical properties in an external magnetic field. The combination of polymers with nano- and micron-sized magnetic materials displays novel and often enhanced properties. The magnetic particles couple the shape of the elastomer to the external magnetic fields. All of the forces acting on the particles are transmitted directly to the polymer chains resulting in either locomotion or deformation. Shape distortion occurs instantaneously and disappears abruptly when external fields are applied or removed, respectively. The combination of magnetic and elastic properties leads to a number of striking phenomena that are exhibited in response to impressed magnetic fields. Giant deformational effects, tuneable elastic modulus, non-homogeneous deformation and quick response to magnetic field open new opportunities for using such materials for various applications.

Elastic materials with tailor-made anisotropy can also be prepared under an external field. The anisotropy manifests itself in both direction dependent elastic modulus and direction dependent swelling. The main purpose of the present chapter is to report on recent advances in the development of magnetic field responsive polymer systems showing the possible application of magnetic fields as new driving mechanisms for such polymers. In the first part of this review we introduce the chemical background of preparation. This is followed by a brief summary of the magnetic properties. The main effects on mechanical properties induced by magnetic fields are discussed next. The mechanical properties under uniform and non-uniform external magnetic fields are described. The next sections are concerned with the kinetics of deformation and with the influence of uniform external magnetic field on the swelling equilibrium. In the final section a comparison is made between electric field and magnetic field responsive gels.

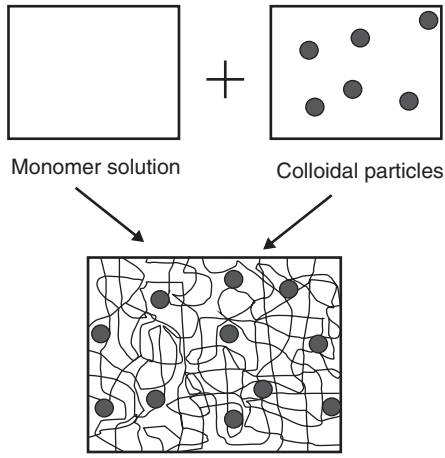
5.2 Preparation of magnetically responsive polymer gels and elastomeric materials

A great number of highly elastic gels and rubbery materials that respond to the presence of magnetic field are chemically cross-linked networks containing magnetic filler particles. Another type of magnetic polymer is represented by core-shell particles, where a magnetic core is covered by a polymer or a polymer gel layer. In the following sections we briefly summarize the preparation procedures with the aid of some representative examples.

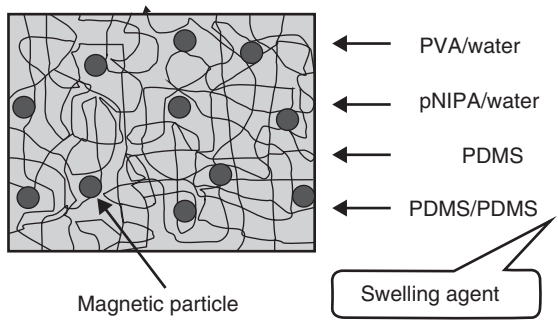
5.2.1 Preparation of magnetic field sensitive gels and elastomers

Magnetic field sensitive gels are chemically cross-linked polymer networks swollen by a ferrofluid (Berkovski and Bashtovoy, 1996; Nakano and Koyama, 1997; Rosenweig, 1985). A ferrofluid or a magnetic fluid is a colloidal dispersion of monodomain magnetic particles with a typical size of about 10 nm.

Preparation of a magnetic field responsive gel requires neither a special polymer nor a special type of magnetic material (Fig. 5.2). For a polymer network one may use very flexible chain molecules, which can be cross-linked. The magnetic filler particles can be prepared from ferro- or ferrimagnetic materials. An important requirement is the strong adsorptive interaction between the solid particles and polymer network chains. One can prepare both magnetic hydrogels or networks swollen by organic ferrofluid. If the swelling agent of the gel is evaporated, then we have magnetic rubber. The magneto-elastic properties of ferrogels can be widely influenced by chemical



5.2 Preparation of magnetic gels.



5.3 Schematic representation of most frequently studied magnetic gels and polymers. PVA = polyvinyl alcohol, pNIPA = poly(N-isopropylacrylamide), PDMS = polydimethylsiloxane.

means. During the preparation it is possible to vary the initial polymer concentration and the degree of cross-linking, as well as the quantity of magnetic particles incorporated into the elastomer. The magnetic properties are determined mainly by the quality and the size of dispersed magnetic material as well as their concentration (Fig. 5.3).

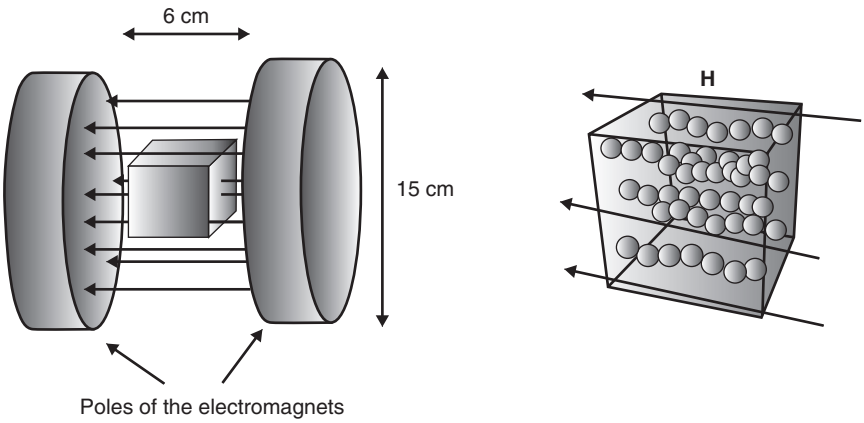
A critical point of the synthesis is the stabilization of solid particles. When mixing colloidal particles with polymer solutions, usually incompatibility occurs. To prevent coagulation and flocculation, stabilization is required. It can be done by either by surfactants or by strong adsorptive interactions of the particles to polymer chains. A highly responsive magnetic gel should have low elastic modulus and high initial susceptibility as well as high saturation magnetization. In order to increase the magneto-elastic response

to external field it is possible to decrease the elastic modulus by swelling. A detailed description of the preparation process can be found in several papers. Zrinyi and coworkers, amongst others, have prepared superparamagnetic magnetite-loaded polyvinyl alcohol hydrogels (Barsi *et al.*, 1996; Filipcsei *et al.*, 2007; Szabo *et al.*, 1998; Zrinyi 1997; Zrinyi *et al.*, 1996, 1997a, 1997b), poly(*N*-isopropylacrylamide) hydrogels (Ghost and Cai, 2010; Xulu *et al.*, 2000) and carbonyl iron- and magnetite-loaded polydimethylsiloxane gels (Varga *et al.*, 2006). Mitsumata and Ohori (2011) have prepared carbonyl iron-loaded polyurethane elastomers. Bajpai and Gupta (2011) reported maghemite- and magnetite-filled polyvinyl alcohol based hydrogels. Choubey and Bajpai (2010) have investigated chemically cross-linked gelatin flooded with iron oxide. Abramchuk *et al.* (2007a, 2007b) investigated several magnetic elastomers. In these works the distribution of the magnetic particles is uniform; therefore the material properties do not depend on the direction of the applied field.

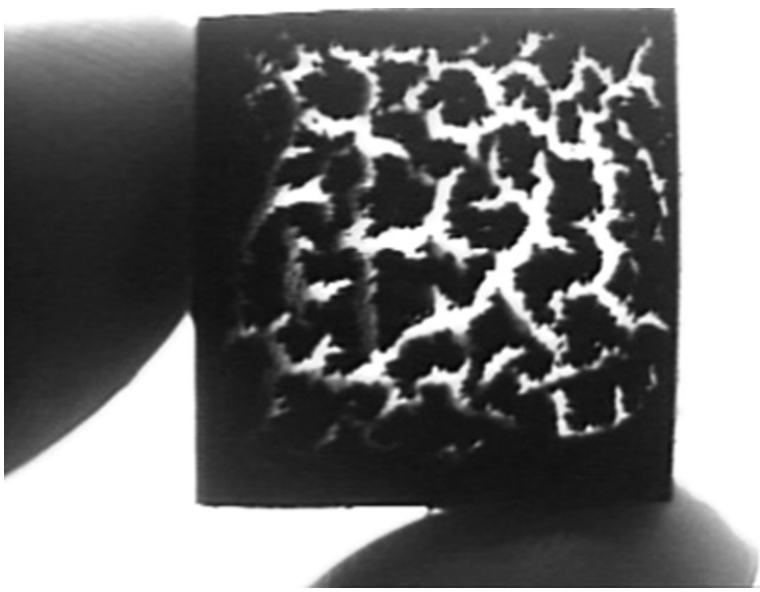
5.2.2 Preparation of anisotropic magnetic field sensitive gels and elastomers

One can prepare magnetic polymers with uniform filler distribution as well as highly anisotropic samples. By utilizing magnetorheological effects (Berkovski and Bashtovoy, 1996; Nakano and Koyama, 1997; Rosenweig, 1985), one can align the magnetic particles and fix the structure by a cross-linking process (Filipcsei, *et al.*, 2007; Varga, *et al.*, 2006). The external magnetic field imposed orients the magnetic dipoles in the mixture and, if the particles are spaced closely enough, mutual particle interactions occur. Owing to the attractive forces, a pearl chain structure develops. The same phenomenon occurs if the liquid is replaced by the monomeric mixture of polymer. If the polymerization reaction is performed under a uniform external field then, due to the mutual interaction between particles, a pearl chain structure develops. The chemical cross-linking locks in the chain-like structure, aligned along the direction of the field. The resulting sample becomes highly anisotropic. A schematic representation of the synthesis is shown in Fig. 5.4.

It is worth mentioning that not only can the magnetorheological effects be used to elicit particle alignment, but also an electrorheological effect can be applied (Tao and Roy, 1994; Zrinyi *et al.*, 2001). Since electric and magnetic fields do not interfere with each other, one can use both fields simultaneously. Depending on the concentration of the magnetic particles, as well as on the applied magnetic field, columnar structures of the magnetic particles built in the elastic matrix can be varied across a wide range. Figure 5.5 shows a poly(dimethyl siloxane) (PDMS) elastomer containing dense columns of pearl chains of magnetic particles.



5.4 Preparation of uniaxially ordered polymer composite under uniform magnetic field.



5.5 Columnar structure of iron particles built in PDMS elastomer.

5.2.3 Evidence of structural anisotropy

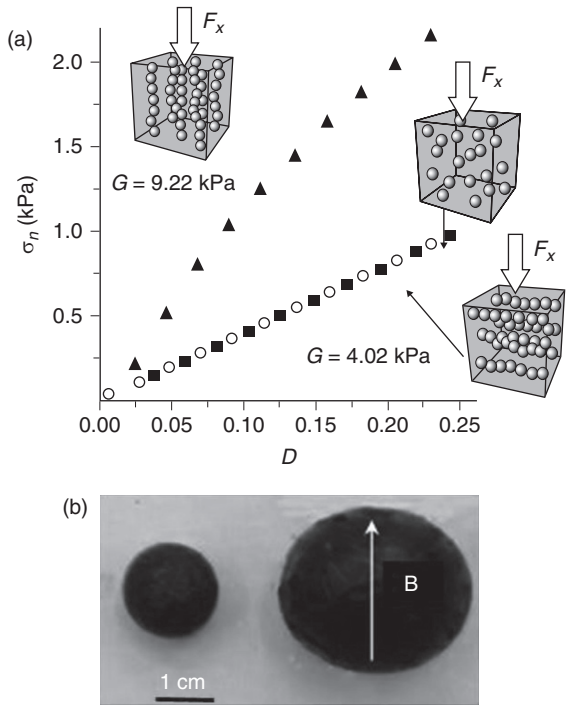
The structural anisotropy manifests itself in both direction dependent elastic modulus and direction dependent swelling. To demonstrate the direction dependent elastic modulus, stress–strain measurements were performed. The elastic modulus was determined by unidirectional compression

measurements and the modulus, G, was calculated on the basis of statistical theory of rubber elasticity (Dusek and Prins, 1969; Mark and Erman, 1988; Treloar, 1975):

$$\sigma_n = G(\lambda - \lambda^{-2}) = GD \qquad [5.1]$$

where σ_n is the nominal stress defined as the ratio of the equilibrium elastic force and the undeformed cross-sectional area of the sample. The deformation ratio, λ, is the length, h, (in the direction of the force) divided by the corresponding undeformed length, h_0. From the plot of nominal stress against $D = -(\lambda - \lambda^{-2})$ data, the slope, G, was calculated by the linear least square method. Figure 5.6a shows the experimental data analysed on the basis of Equation [5.1]. It is seen in the figure that the slope of the straight lines which provide the elastic modulus, G, is direction dependent. The elastic modulus was found to be largest if the compression force and the direction of the pearl chain structure are parallel. In the perpendicular case we have found a much smaller modulus. This finding indicates a strong mechanical anisotropy. Evidence is also provided by the equilibrium swelling degree. According to the thermodynamics of swelling, the elastic modulus and the degree of equilibrium swelling are interrelated (Dusek and Prins, 1969; Mark and Erman, 1988; Treloar, 1975). The higher the modulus, the smaller the equilibrium swelling degree. The anisotropic filler-loaded PDMS sample was put into cyclohexane at room temperature. Cyclohexane is a good solvent for PDMS and therefore the network begins to swell. The swelling degree increased and it was found that the swelling degree parallel to the chain-like particle orientation is less than in the perpendicular case. This finding is evidenced by Fig. 5.6b.

Figure 5.7 also shows the effect of particle arrangement on the elasticity of magnetite-loaded PDMS gel. The magnetite content of this gel is 40 wt%. Three different states of the gel are shown. At the left of Fig. 5.7a, the sample is stress-free. In the middle, a load of 50 g is placed on the top surface of the gel. In this case the mechanical stress due to the weight is parallel to the particle arrangement indicated by the arrow. The right-hand side of Fig. 5.7a shows the same gel averted by 90°. As a result, the direction of mechanical stress and the direction of particle alignment are perpendicular and the same load results in higher deformation. Figure 5.7b provides the stress–strain curve for the direction dependent mechanical experiments. It is seen in the figure that the slope which provides the elastic modulus is very different for the two cases. The gel shows softer mechanical behaviour when the direction of strain and that of particle arrangement are not parallel. It also shows that the elastic modulus for the gel containing the same amount of randomly distributed particles is between these two situations.

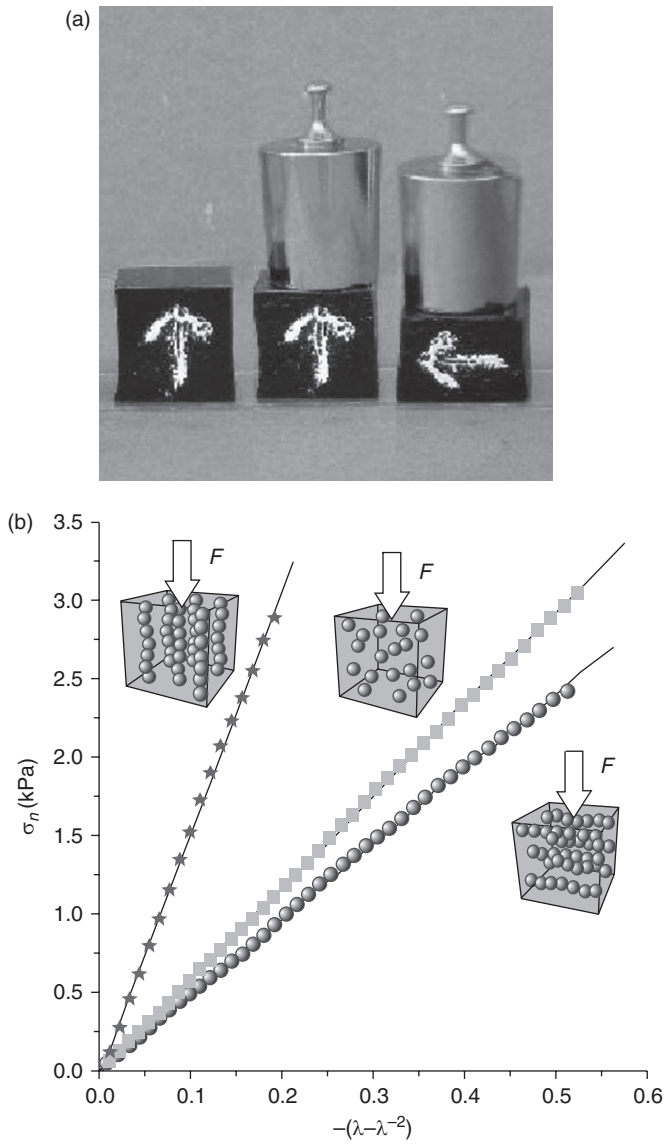


5.6 Mechanical measurement performed on the cube shaped, magnetite-loaded PDMS gel (a). The concentration of the iron oxide particles in the gel was 30 wt% and the cross-linker content was 3 wt%. The value of the elastic modulus, *G*, is indicated on the figure. Anisotropic swelling of magnetite-loaded PDMS in cyclohexane (b). The arrows indicate the direction of the magnetic field (particle chains) during the preparation.

Figure 5.8 shows the effect of structural anisotropy on swelling. The anisotropic filler-loaded PDMS dry network was put into n-hexane, which is also a good solvent for PDMS. According to the thermodynamics of swelling, the elastic modulus and the equilibrium swelling degree are interrelated. The higher the modulus, the smaller the equilibrium swelling degree. It was found that the swelling degree parallel to the chain-like particle orientation is less than in the perpendicular case. This finding is shown by Fig. 5.8.

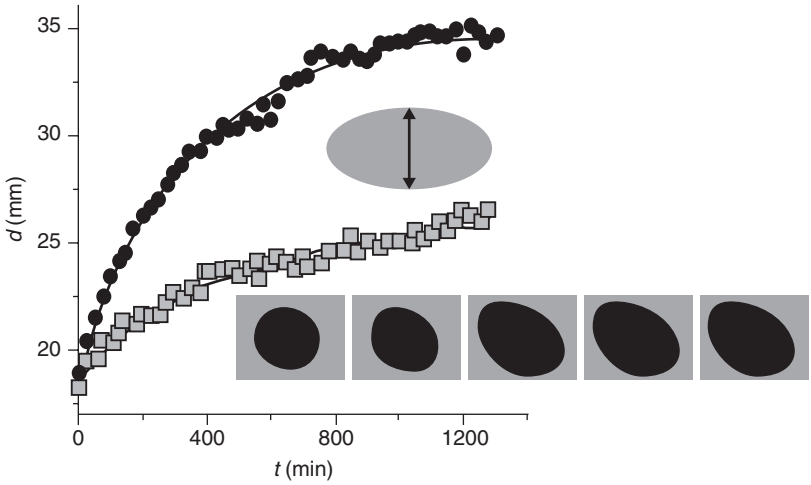
5.2.4 Preparation of magnetic field sensitive core-shell type particles

Preparation of core-shell type magnetic particles comprises two steps. First, a magnetic core has to be prepared; the core can be either magnetic nano-particle

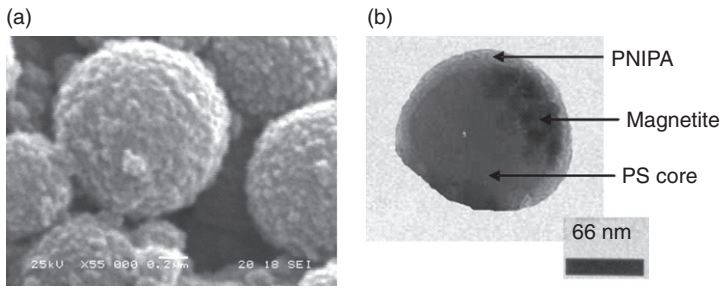


5.7 Anisotropic mechanical behaviour as seen by the naked eye (a) and by stress–strain measurements (b). Three samples are compared, each having the same amount of filler particles, but with different particle distribution as indicated in the figure. In figure (a) the arrows indicate the chain alignment.

or polymer bead containing dispersed magnetic particles. Lien and coworkers used monodisperse magnetite particles encapsulated by tetraethyl orthosilicate (SiO_2/Fe_3O_4) as core and then grafted poly(*N*-isopropylacrylamide) (Lien and Wu, 2008). The result was a magnetic field and temperature



5.8 Anisotropic swelling of a magnetic PDMS gel in n-hexane.



5.9 Transmission electron microscopy (TEM) picture of magnetic PS particles (a) and core-shell type of MPS-pNIPA particles in the dry state (b). According to the TEM pictures, the average thickness of the dry pNIPA layer was found to be 8.8 nm.

responsive particle. Another type of two-functional particle was prepared by Xulu and coworkers (Xulu *et al.*. 2000). Magnetic polystyrene latex (MPS) with an average size of 90 nm was prepared by the miniemulsion technique. This particle was considered to be a core of polymer gel layer. The second step was the shell polymerization process in which a gel layer of different thickness was grafted onto the surface of MPS particles. The core contains the magnetic nanoparticles, and the shell can be made of any type of gel. Poly(*N*-isopropylacrylamide) hydrogel, abbreviated as pNIPA gel, is one of the most frequently studied temperature responsive gels (Fig. 5.9). It exhibits a remarkable volume change in response to temperature changes. The transition temperature above which the network chains are in the collapsed state is called the lower critical solution temperature (LCST). For pNIPA

gels swollen in water, the LCST has been found to be 34°C. There are several other gels showing reversible swelling and shrinking transition with different LCST or upper critical solution temperature (UCST). These gels are often used to immobilize enzymes and as carriers of certain functional groups important for biochemical or biomedical applications (De Rossi *et al.*, 1991; Okano *et al.*, 1998; Nobuhiko *et al.*, 2004).

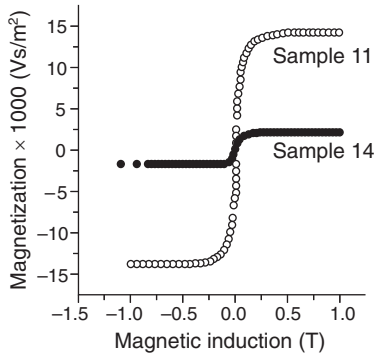
5.3 Magnetic properties of filler-loaded polymers

In both magnetic gels and magnetic rubbers the solid nanoparticles are the elementary carriers of a magnetic moment. These fine particles have properties that are significantly different from those of the corresponding bulk materials (Berkovski and Bashtovoy, 2006; Dormann and Fiorani, 1992; Hadijipanayis and Seigel, 1994; Rosenweig 1985). In many cases carbonyl iron [Fe] and iron oxide particles, [Fe_2O_3 and Fe_3O_4] are used as magnetic filler particles.

The magnetic properties of γ-Fe_2O_3 powder and magnetite [Fe_3O_4] nanoparticles are quite different. γ-Fe_2O_3 is a magnetically hard material showing a hysteresis loop on the magnetization curve. The shape of the hysteresis curve is dependent upon the hardness of the material as well as the magnitude and rate of the change of applied field. Hysteresis makes permanent magnetism possible, but it also represents an energy loss mechanism producing heat. Fe_3O_4nanoparticles with a typical size of 10 nm are magnetically soft materials. They exhibit superparamagnetic behaviour.

The monodomain ferromagnetic particles of colloidal size are the elementary carriers of a magnetic moment in the magnetic gels. In the absence of an applied field they are randomly oriented due to thermal agitation, and thus the ferrogel has no net magnetization. As soon as an external magnetic field is applied, the magnetic moments tend to align with the field to produce a bulk magnetic moment, M. With ordinary field strengths the tendency of the dipole moments to align with the applied field is partially overcome by thermal agitation. As the strength of field increases, all particles eventually align their moments along the direction of field and, as a result, the magnetization saturates. If the applied field is turned off the particles quickly randomize and magnetization is again reduced to zero. This means that the magnetization curve shows no hysteresis at all and can be fitted by a Langevin function corrected with the distribution of magnetic dipole moments.

If we suppose that monodisperse nano-sized (superparamagnetic) particles are randomly distributed in the polymer network, and the magnetization of individual particles in gel equals the saturation magnetization of the pure and bulk corresponding material, M_s, which can be described by the Langevin function (Berkovski and Bashtovoy, 2006; Hadijipanayis and Seigel, 1994; Restorf, 1994):



5.10 Magnetization curve of two magnetic poly N-isopropyl acrylamide (MNIPA) gels containing different amounts of magnetic particles. The concentration of magnetite in sample 11 is 23.1 wt %, whereas sample 14 contains 3.3 wt % of magnetite.

$$M = \phi_m M_s L(\xi) = \phi_m M_s \left(\coth \xi - \frac{1}{\xi} \right) \qquad [5.2]$$

where ϕ_m stands for the volume fraction of the magnetic particles in the whole gel, and the parameter ξ of Langevin function, $L(\xi)$ is defined as

$$\xi = \frac{mH}{k_B T} \qquad [5.3]$$

where H represents the strength of an external magnetic field, m is the magnetic moment of subdomain particles, k_B denotes the Boltzmann constant and T is temperature.

On the basis of Fig. 5.10, we can conclude that, within the experimental accuracy, no hysteresis loop has been observed. This finding means that no permanent magnetization takes place in magnetic pNIPA gels at room temperature. This is an important result which means that, in alternating magnetic field, the transformation of magnetic into thermal energy is rather small.

5.4 Elastic behaviour of magnetic gels and elastomers

In the absence of an external magnetic field a ferrogel presents a mechanical behaviour very close to that of a swollen filler-loaded network. Since a typical magnetic gel can be considered a dilute magnetic system, we may neglect the influence of magnetic interactions on the modulus. Thus the stress–strain

dependence of an unidirectional deformed gel sample can be described by Equation [5.1]. The modulus of the magnetic gel can be expressed as a function of filler concentration, φ_m:

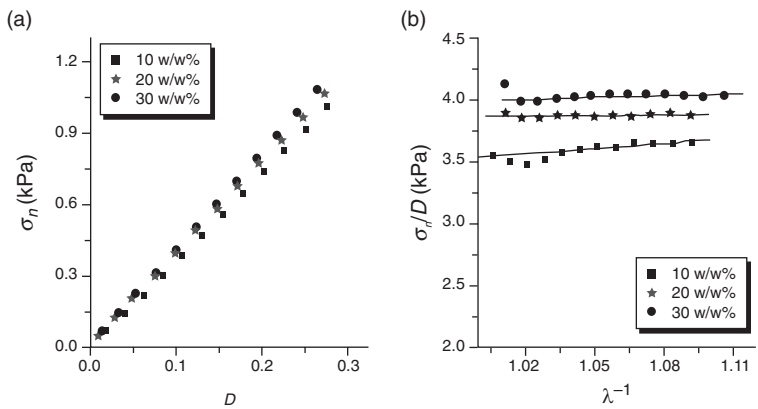
$$G = G_o\left(1 + k_E \varphi_m\right) \qquad [5.4]$$

where G_o denotes the modulus of gel without colloidal filler particles and k_E is the Einstein-Smallwood parameter. For non-interacting spherical particles $k_E = 2.5$. Equation [5.4] describes the reinforcement effect due to the filler–polymer interaction. The modulus of a magnetic gel can be varied by the cross-linking density through G_o and by the concentration of colloidal particles via φ_m. It must be mentioned that in many cases Equation [5.1] cannot be used to fit the experimental data. Deviation from the Gaussian theory of rubber elasticity may be due to finite chain extensibility and entanglement effects (Dusek and Prins, 1969; Kilian, 1987; Mark and Erman, 1988; Treloar, 1975) and often represented by the Mooney plot:

$$\frac{\sigma_n}{\lambda - \lambda^{-2}} = C_1 + C_2 \lambda^{-1} \qquad [5.5]$$

where C_1 and C_2 are constants and the modulus is $G = C_1 + C_2$.

In order to test the applicability of Equations [5.1] and [5.5] for gels filled with magnetic particles, a Mooney-Rivlin representation of experimental data obtained in the absence of external magnetic field is shown in Fig. 5.11.



5.11 Stress–strain measurements for PDMS samples filled with randomly distributed carbonyl iron particles. (a) On the basis of statistical theory of rubber elasticity and (b) Mooney-Rivlin representation of experimental data. Symbols represent different amounts of iron particles as indicated in the figure.

It may be seen that the nominal stress divided by $\lambda - \lambda^{-2}$ seems to be slightly dependent on the strain.

5.4.1 Origin of force generated by the magnetic field

In order to determine the nature of the force generated by a polymer gel, one must consider all the relevant interactions that contribute to the force or displacement. We learned from the thermodynamics of rubber elasticity that the molecular mechanism of force generation in the network chains is made up of two different contributions. In general, energetic and entropic effects must be taken into account:

$$f = \frac{\partial A}{\partial l} = \frac{\partial U}{\partial l} - T\frac{\partial S}{\partial l} = f_U + f_S \qquad [5.6]$$

where A, U and S represent the free energy, internal energy and entropy, respectively. f_U and f_S denote the derivative of internal energy and entropy with respect to displacement, l, in order to yield force components. On the basis of Equation [5.6] one can classify the gels:

- Swelling or shrinking of polymer gels is due to imbalance between osmotic and entropic forces. The mechanical stress is generated mainly by continuous conformational changes, or volume-phase transition of either LCST or UCST type (Tanaka. 1978, 1982). The conventional polymer gels which swell or shrink as a consequence of certain stimuli, can be considered entropy-driven responsive gels.
- The new generation of composite gels exploits energetic effects to create force or work. These gels are either composite gels or networks swollen by a liquid that can change its dielectric properties drastically under an external field. These kind of gels are known as energy-driven responsive gels because the network chains deform as a result of strong energetic interactions.

There are significant differences when comparing the mechanism of force generation occurring in responsive gels of these two kinds. In the case of entropy-driven gels, the force is due to volume change, whereas for energy-driven polymer gels there is no volume change, but shape change occurs. Consequently, the response rate is also very different. Electric or magnetic polarization that induces the deformation of field responsive gels is much faster than swelling or shrinking, which are controlled by much slower diffusion mechanisms. Another substantial difference lays in the nature of the response. In the case of energy-driven gels, both the stimulus and the

response are directed, whereas in the case of volume change, it is difficult to localize the volume change to certain part of the gels, and hence to produce directed motion.

5.4.2 Response of magnetic gels to uniform magnetic field

Recently, there has been considerable interest in soft materials with uniform magnetic field-induced deformation (Bacri *et al.*, 1982, 1983; Dean *et al.*, 2011; Patel *et al.*, 2008).

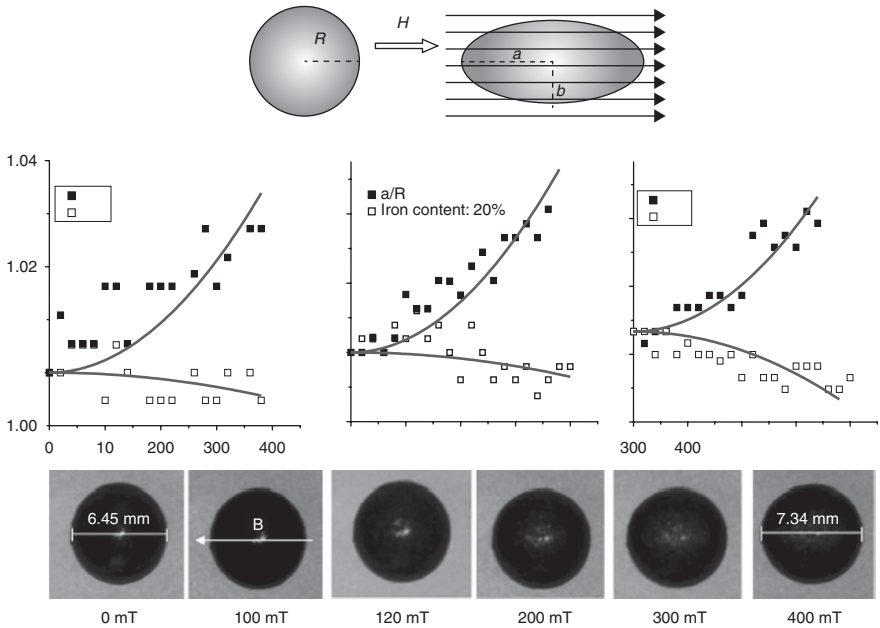
Evolution of the shape of ferrofluid magnetic drops in the presence of a magnetic field has also been reported. Dynamic response of a ferrogel under a sudden change of uniform magnetic field has been reported (Gollwitzer *et al.*, 2008). It was found that the time dependence of the elastic shear modulus causes elongation to increase with time and the rapid excitation causes the ferrogel sphere to vibrate. The phenomena were described by the theory of Raikher and Stolbov (2003, 2005).

Similar to conventional magnetostrictive materials, ferrogels elongate in uniform fields. We have studied the effect of uniform magnetic field on the shape of carbonyl iron-loaded PDMS gels. In this work it was assumed that the shape of a ferrogel is controlled by competition between magnetic and elastic energy. For a constant volume of ferrogel bead and a given magnetic field, the shape of the gel can be obtained by minimization of the total energy with respect to the aspect ratio (a/R). This process yields

$$\frac{a}{R} = 1 + \kappa_a \cdot \frac{\chi_m^2 H^2}{G} \qquad [5.7]$$

$$\frac{b}{R} = 1 + \kappa_b \cdot \frac{\chi_m^2 H^2}{G} \qquad [5.8]$$

where χ_m denotes the initial susceptibility, κ_a and κ_b are the magneto-deformational susceptibility parallel and perpendicular to the magnetic field, respectively. For an incompressible material, $\kappa_a = 1/15$ and $\kappa_b = -1/30$. Equations [5.7] and [5.8] predict that the sphere elongates along the magnetic field lines and the measure of elongation is related to the square of magnetic field intensity. It is also seen that the higher the elastic modulus, the weaker the magneto-deformation effect. The experimental results preformed on carbonyl iron-loaded PDMS gels support these ideas as shown in Fig. 5.12. There, one can see magnetic gels do not become significantly deformed in uniform fields. The reason for this is that the small



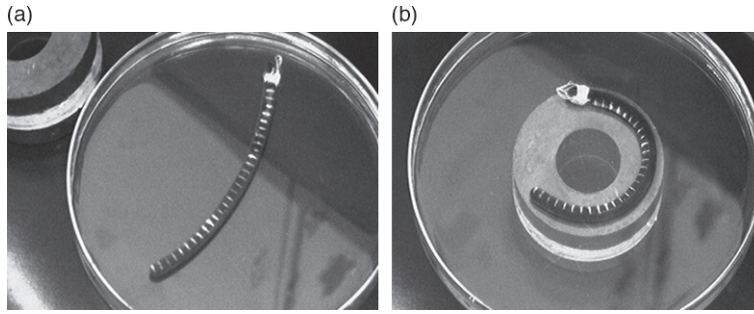
5.12 The dependence of aspect ratio on the external magnetic field intensity. (Top) The sphere elongates along the magnetic field lines; (middle and bottom) the dependence of a specific ratio on the external magnetic field intensity. The solid lines represent the square law dependence of aspect ratio on field intensities. ■ indicate the deformation parallel to the field direction, whereas the □ stand for the perpendicular direction.

superparamagnetic particles embedded in the polymer matrix experience no force in a uniform magnetic field.

5.4.3 Response of magnetic gels to non-uniform magnetic field

When magnetic gel beads are placed into a spatially non-uniform magnetic field, forces act on the magnetic particles, and the magnetic interactions are enhanced. The stronger field attracts the particles and, owing to their small size and strong interactions with molecules of dispersing liquid and polymer chains, they all move together. The magnetic field drives and controls the displacement. The force density, f_m, on a piece of ferrogel can be generally written as

$$f_m = [M\nabla]H \qquad [5.9]$$



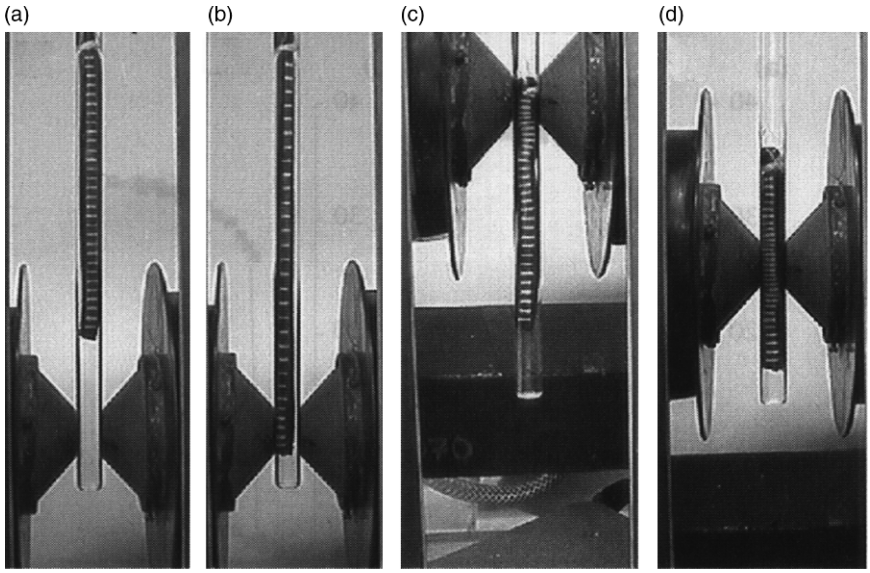
5.13 Shape distortion of a ferrogel, due to non-uniform magnetic field produced by a permanent magnet: (a) the magnetic PVA gel is placed 15 cm away from the magnet; (b) the magnetic PVA gel is placed on a permanent magnet that induces shape change.

where ∇ represents the gradient operation. The orientation of f_m is parallel to the direction of the magnetic field.

In a uniform magnetic field a ferrogel experiences no net force. When it is placed in a spatially non-uniform magnetic field, forces act on the superparamagnetic particles, and the magnetic interactions are enhanced. Because of the cross-linking bridges in the network, changes in molecular conformation can accumulate and lead to macroscopic shape changes and motion. The principle of the shape transformation and motility of the ferrogel lies in a unique magneto-elastic behaviour. The magnetic field drives and controls the motion, and the balance of magnetic and elastic interactions sets the final shape.

If a ferrogel is placed in a non-uniform magnetic field, as shown in Fig. 5.13, depending on the field distribution in space, curvature may occur. The gel has its original (straight) shape if the Petri dish containing the ferrogel is far above the magnet. In this case the magnetic field strength is so small that the magnetic force is too weak to deform the gel. Due to the enhanced magnetic interaction, curvature immediately occurs when the Petri dish is placed on the magnet. Not only curvature, but also stretching and contraction, can be realized in ferrogels in inhomogeneous magnetic field. The ferrogel can be made to repeatedly bend and straighten many times without damaging the gel. The ability of ferrogels to undergo successive bending and stretching can be used to construct new types of soft actuators as well as worm-like motions.

Let us consider in the Cartesian coordinate system a non-uniform magnetic field the strength of which varies only in the direction of z. The measure of this variation is characterized by the gradient of the magnetic field in space. This field can be produced by an electromagnet, and the steady

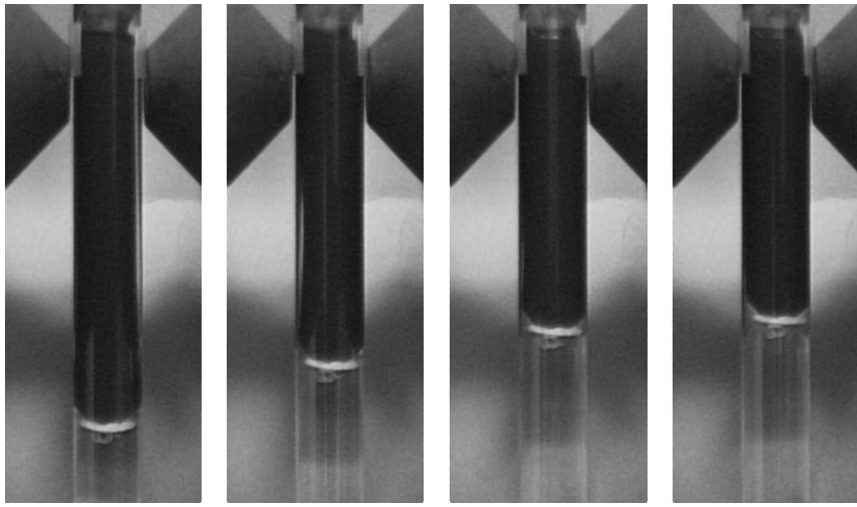


5.14 Elongation and contraction of a ferrogel in non-uniform magnetic fields: (a) no magnetic field is applied; (b) the maximal field strength is located under the lower end of the gel; (c) the maximal field strength is located on the top surface of the ferrogel; (d) the maximal field strength is focused in the middle of the gel along its axis.

current flowing through the electromagnet determines the dependence of the field strength on coordinate z. Let us suspend vertically (parallel to z) a cylindrical gel sample. When it is subjected to a non-uniform magnetic field, a tensile stress develops in the z direction. Due to this stress – and depending on the sign of the field gradient (experimental arrangement) – elongation or contraction may occur. This is demonstrated in Fig. 5.14 where a ferrogel is shown in different types of magnetic fields produced by two plane-parallel poles of electromagnets. The position of the poles of electromagnets can be varied along the z direction.

We present here four different situations: (a) no magnetic field is applied; (b) the axis of magnetic poles is below the lower end of the gel; (c) the axis of magnetic poles is at the top surface of the ferrogel; and (d) the axis of the poles is in the middle of the gel along z. In case (a) no deformation occurs. In the presence of an applied magnetic field, a field gradient develops parallel to the gel axis and results in elongation in case (b) and contraction at arrangements denoted by (c) and (d).

We have studied the unidirectional shortening of ferrogel samples excited by non-homogeneous magnetic fields. A cylindrical gel sample was suspended in water vertically between plane-parallel poles of an

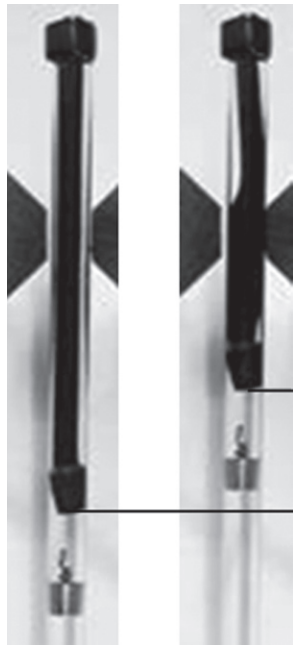


5.15 Contraction without load induced by different magnetic field strengths.

electromagnet. The position of the top surface of the gel was fixed and the highest field strength was located at this point. The steady current intensity in the solenoid-based electromagnet was varied in order to produce different magnetic field distributions. The maximum magnetic field strength (magnetic flux density) of 300 mT developed at the upper part of the gel and disappeared within 120 mm along the axis of the cylindrical gel sample. It is worth mentioning that 300 mT is a field strength which is less than the field strength measured at the surface of common permanent magnets. Due to the field gradient directed from bottom to top along the gel axis, contraction occurs. The measure of this atonic (no-load) contraction strongly depends on the structure of the magnetic gel as well as on the distribution of magnetic field along the axis of the gel. Since the highest magnetic field strength can be controlled by the intensity of the steady current flowing through the electromagnet, it is possible to realize different degrees of contraction as shown in Fig. 5.15.

One can establish that significant contraction can be induced by moderate magnetic field gradient. The contractile activity of magnetic gels can be used to lift a load that is to produce work as shown in Fig. 5.16.

In a non-accelerating system the force density manifests itself as a stress distribution which must be balanced by the network elasticity. A completely balanced set of forces is, in this respect, equivalent to no external force at all. However, they affect the gel internally tending to change its shape or size or both. In general, the deformation induced by a magnetic



5.16 Lifting a load by contraction of magnetic gel.

field cannot be considered a homogeneous deformation, since the driving force, $M\nabla H$ varies from point to point in space. However, one can find a special distribution of magnetic field where the deviation from the homogeneous case is not significant. We have derived the basic magneto-elastic equation for uniaxial deformation with a non-uniform magnetic field (Zrinyi *et al.*, 1996, 1997):

$$\lambda_H^3 - \beta\left(H_h^2 - H_m^2\right)\lambda_H - 1 = 0 \qquad [5.10]$$

where λ_H represents the deformation ratio due to magnetic interactions only [no load], H_h and H_m denote the magnetic field strength at the bottom and at the top of a ferrogel cylinder, β is a parameter defined as

$$\beta = \frac{\mu_0 \chi}{2G} \qquad [5.11]$$

which includes the initial susceptibility, χ, as well as the elastic modulus, G, of the magnetic gel.

Equation [5.10] was derived by considering homogeneous deformation and a linear relationship between magnetization and magnetic field strength. It was also assumed that the Gaussian network behaviour can be used as an approximation. This equation says that if we suspend a magnetic gel in a non-homogeneous magnetic field in such a way as $H_h > H_m$, then elongation occurs. In the opposite case, where $H_h < H_m$, a contraction is predicted. It is rather difficult to achieve an analytical solution of Equation [5.10] since during deformation the position of the bottom of the gel keeps on changing and therefore H_h depends on λ_H.

5.5 Kinetics of shape change

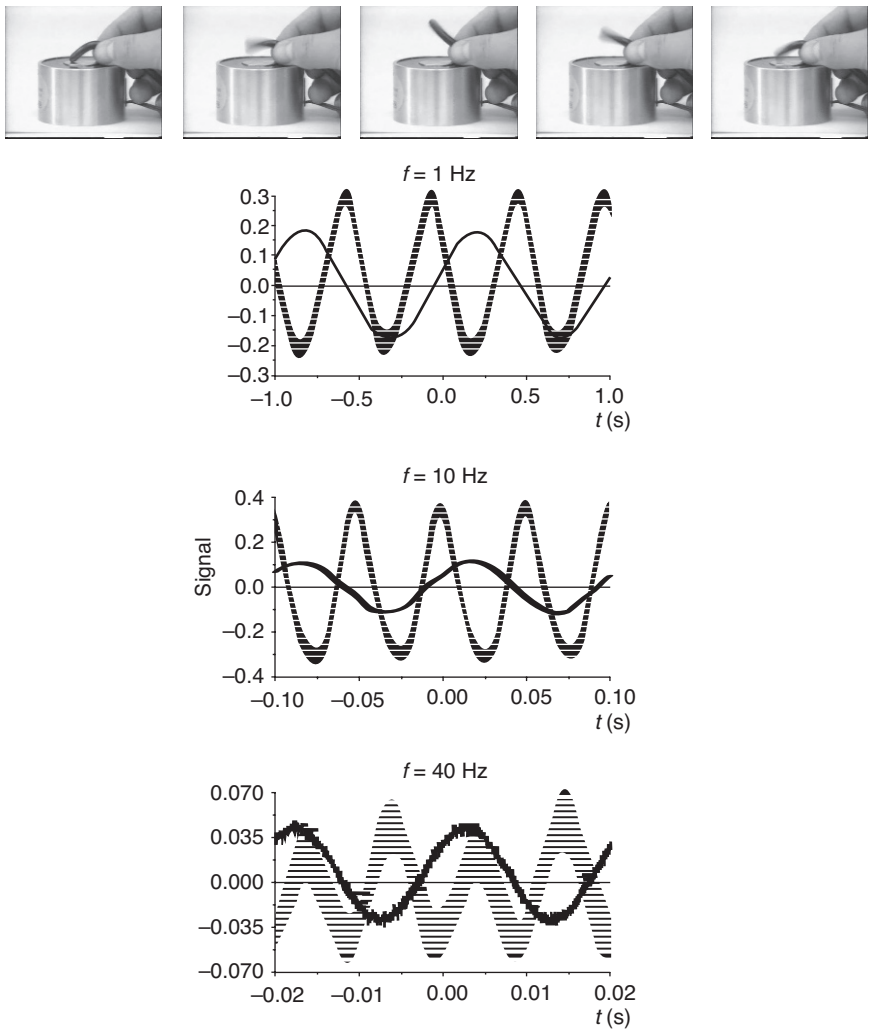
Any technical application of magnetic field sensitive polymer gels critically depends on their response time. It is therefore of primary interest to investigate the deformation behaviour of ferrogels in response to the magnetic stimulus. Since the magnetic field is created by electromagnets, it is easy to achieve dynamic conditions by modulating current intensity. We applied stepwise and sine wave modulation using a function generator in the frequency range 0.01 to 100 Hz. A cylindrical gel sample characterized by a height of 8 mm and radius of 4.5 mm was put onto the upper surface of a standing electromagnet. The position of the top surface of the gel was measured with a light beam and the displacement was monitored with the aid of a light diode as voltage signal. Due to the experimental arrangement, a contraction takes place when current flows through the solenoid. When the current is turned off the gel expands and its equilibrium shape is recovered. We measured the elastodynamic response of ferrogels at different frequencies. It is seen in Fig. 5.17 that the ferrogel shows reversible shape change in response to magnetic field modulation. A fast on–off switching of the steady current results in fast contraction–extension cycles.

It is also seen that the gel repeats the contraction–extension cycles in response to stepwise magnetic field changes. Neither phase shift nor magneto-elastic relaxation can be observed at this frequency within the experimental accuracy.

More recently, dynamic response of a ferrogel under sudden change of uniform magnetic field has been reported (Gollwitzer *et al.*, 2008).

5.6 The swelling equilibrium under a uniform magnetic field

A comprehensive study of the effects of a uniform field on swelling behaviour is still lacking. It is therefore a major objective of this work to build a



5.17 Elastodynamic response of a ferrogel at different frequencies of sine wave modulation. (Top) Photographs about a ferrogel with elastodynamic response. Solid line represents the modulated current, dotted line corresponds to the elastic response. The frequency, *f*, is indicated on the figure.

significant understanding of the swelling behaviour of ferrogels under the action of uniform external magnetic field (Filipcsei *et al.*, 2009, 2010). In the absence of an external magnetic field, a ferrogel presents a swelling behaviour very close to that of a swollen filler-loaded network. The chemical potential of the swelling agent (denoted by index 1), $\Delta\mu_1$, can be expressed

as the sum of mixing, $\Delta\mu_{1,\text{mix}}$, and elastic, $\Delta\mu_{1,\text{el}}$, contributions (Dusek and Prins, 1969; Mark and Erman, 1988; Zrinyi *et al.*, 1982):

$$\Delta\mu_1 = \Delta\mu_{1,\text{mix}} + \Delta\mu_{1,\text{el}} \qquad [5.12]$$

These quantities can be derived from free energy of the elastic and mixing interactions.

$$\Delta\mu_{1,\text{mix}} = RT\left[\ln\left(1-\Phi_P\right)+\Phi_P+\chi_H\Phi_P^2\right] \qquad [5.13]$$

$$\Delta\mu_{1,\text{el}} = RTA\nu^* q_o^{-2/3}\Phi_P^{1/3} \qquad [5.14]$$

where Φ_P represents the volume fraction of the polymer in the gel, χ_H stands for the Huggins interaction parameter and ν^* is the concentration of the elastically active network chains in the dry state. A is used as a model parameter with a value of 1 or ½. R and T are the gas constant and temperature, respectively.

Figure 5.18 shows the dependence of chemical potentials $\Delta\mu_{1,\text{mix}}$, $\Delta\mu_{1,\text{el}}$ and $\Delta\mu_1$ on the volume fraction of the polymer. In equilibrium with pure solvent, Equation [5.1] can be written as $\Delta\mu_1 = 0$. Thus:

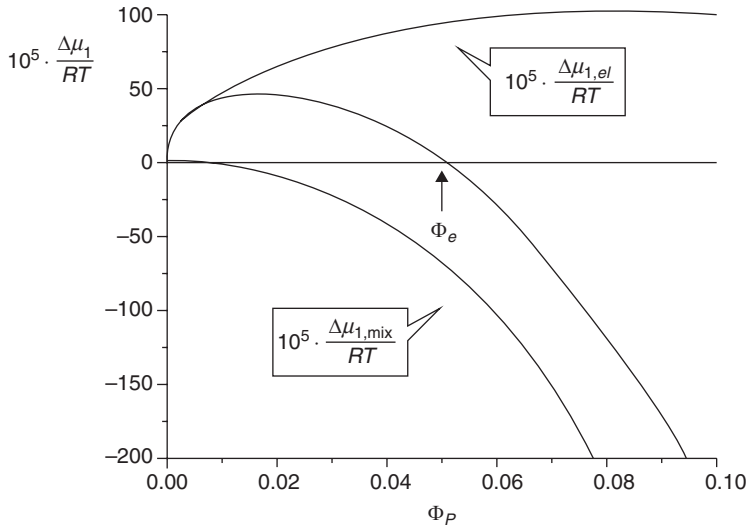
$$RT\left[\ln\left(1-\Phi_e\right)+\Phi_e+\chi_H\Phi_e^2\right]+RTA\nu^* q_o^{-2/3}\Phi_e^{1/3} = 0 \qquad [5.15]$$

where Φ_e denotes the volume fraction of the polymer in swelling equilibrium. The solution of Equation [5.4] for Φ_e gives the dependence of swelling degree $[q_V = 1/\Phi_e]$ on different quantities, like $\chi_H(T)$ and ν^*.

A description of the effect of magnetic field on the thermodynamic properties requires the adoption of the magnetic energy as additional interaction energy. We consider here a piece of ferrogel under the action of a homogeneous magnetic field. The magnetic induction, B, the magnetic field strength, H, and the magnetic moment per unit volume, m, are all parallel. The Gibbs free energy can be expressed as:

$$dG = Vdp - SdT + \sum_i \mu_i dn_i + \mu_o HdM \qquad [5.16]$$

where $M = V \cdot m$ which is the total magnetic moment in the gel of volume V.



5.18 Components of the chemical potential of the swelling agent as a function of volume fraction of the polymer. For the calculation $\chi_H = 0.3$ and $A\nu^* q_o^{-2/3} = 2.15 \cdot 10^{-3}$ was used.

In order to study the effect of the external magnetic field on the swelling equilibrium we rewrite Equation [5.13] by introducing a new function $G - \mu_o HM$, which is a Legendre transformation of the Gibbs free energy function of G.

$$d\left(G - \mu_o HM\right) = Vdp - SdT + \sum_i \mu_i dn_i - \mu_o MdH \qquad [5.17]$$

We also assume that the saturation magnetization occurs at very high magnetic field intensities. Taking into account Equation [5.14] with constant temperature and pressure, and low magnetic field intensities, a Maxwell relation gives

$$\left(\frac{\partial \mu_1}{\partial H}\right)_{T,P,n_2} = -\mu_o VH\left(\frac{\partial \chi_m}{\partial n_1}\right)_{T,P,H,n_2} \qquad [5.18]$$

where χ_m represents the molar magnetic susceptibility and the subscript 1 stands for the swelling agent. The magnetic susceptibility of ferrogel samples was found to be linearly dependent on the concentration of magnetic particles.

$$\chi_m = k_\chi \Phi_m = k_\chi \frac{v_m}{v_p} \Phi_P \qquad [5.19]$$

where Φ_m stands for the volume fraction of the magnetite in the whole gel, and v_m and $v_p$denote the volume of the polymer and the magnetic material in the gel, respectively. The quantity k_χ was found to be 0.338 for magnetite-loaded hydrogels (Zrinyi *et al.*, 1999).

The quantities in Equation [5.15] [$V, n_1$and χ_m] can be related to the volume fraction Φ_P of the polymer in the gel.

$$V\left(\frac{\partial \chi_m}{\partial n_1}\right)_{T,P,H,n_2} = -k_\chi V_1 \frac{v_m}{v_p} \Phi_P \qquad [5.20]$$

where V_1 denotes the partial volume of the swelling agent.

Combining Equations [5.15] and [5.17] results in

$$\left(\frac{\partial \mu_1}{\partial H}\right)_{T,P,n_2} = \mu_o k_\chi V_1 \frac{v_m}{v_p} \Phi_P H \qquad [5.21]$$

where μ_1 here represents the magnetic contribution of the chemical potential of ferrofluid. After integration we have for the magnetic contribution of the swelling agent:

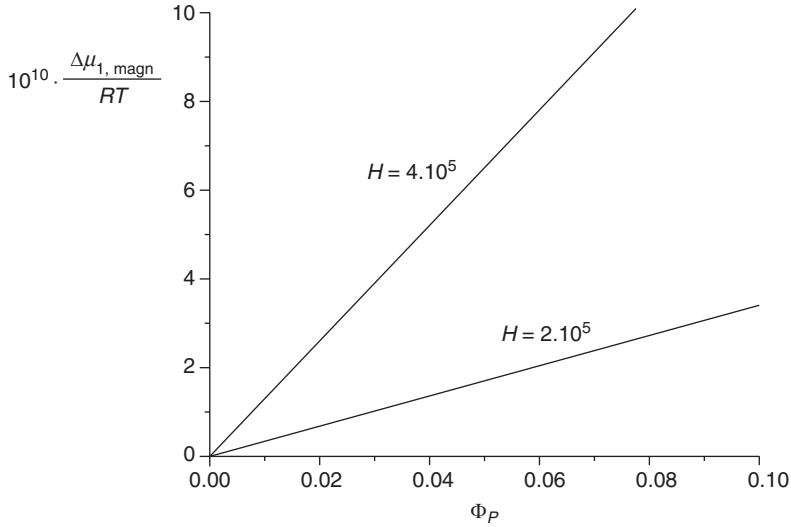
$$\Delta\mu_{1,\text{magn}}\left(\Phi_p, H\right) = \frac{1}{2}\mu_o k_\chi V_1 \frac{v_m}{v_p} \Phi_P \cdot H^2 \qquad [5.22]$$

This equation says that the magnetic interaction increases the chemical potential of the swelling agent. A linear dependence of $\Delta\mu_{1,\text{magn}}$ on the volume fraction of the polymer has been obtained, as shown in Fig. 5.19.

The dependence of the chemical potential of the swelling agent on the network parameter and on the magnetic field strength can be expressed as:

$$\frac{\Delta\mu_1}{RT} = \left[\ln\left(1-\Phi_P\right) + \Phi_P + \chi_H \Phi_P^2\right] + A\nu^* q_o^{-2/3} \Phi_P^{1/3} + \frac{\mu_o k_\chi V_1}{2RT} \frac{v_m}{v_p} \Phi_P \cdot H^2 \qquad [5.23]$$

Figure 5.20 shows the effect of magnetic field intensity on the dependence of $\Delta\mu_1$on the polymer concentration.



5.19 Dependence of magnetic chemical potential on the volume fraction of the polymer at two field intensities given in the figure in A/m units. For the calculation $\mu_o k_\chi V_1 v_m / 2RTv_p = 8 \cdot 10^{-10}$ was used.

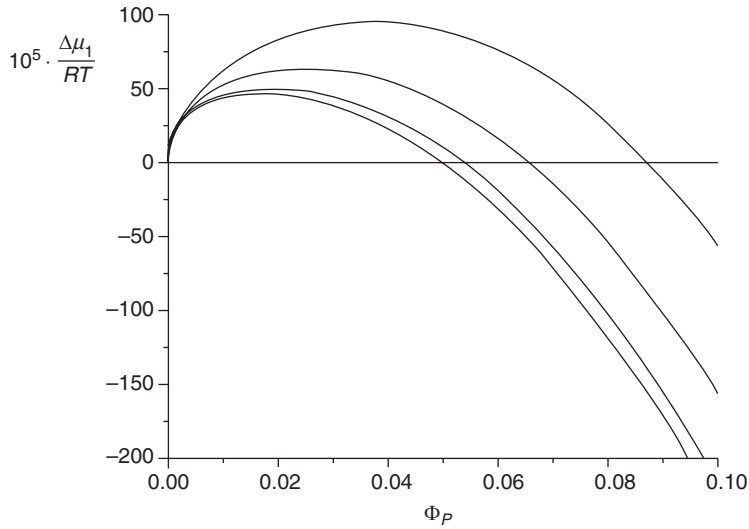
The condition of swelling equilibrium under uniform magnetic field can be expressed as follows:

$$\Delta\mu_1 = \Delta\mu_{1,\mathrm{mix}} + \Delta\mu_{1,\mathrm{el}} + \Delta\mu_{1,\mathrm{magn}} = 0 \qquad [5.24]$$

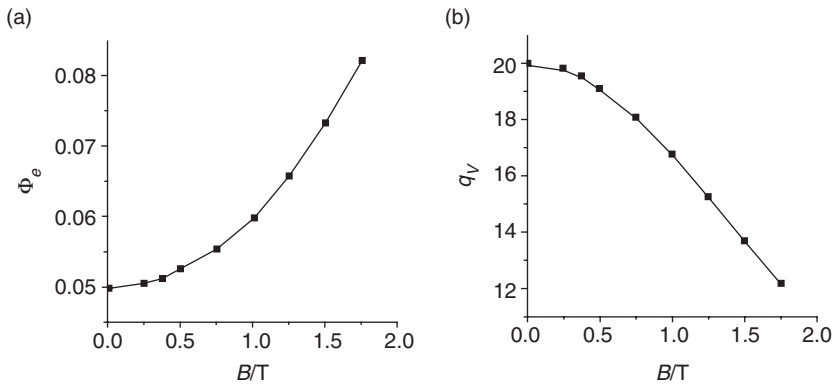
$$\ln\left(1-\Phi_e\right) + \Phi_e + \chi_H \Phi_e^2 + A\nu^* q_o^{-2/3} \Phi_e^{1/3} + \frac{\mu_o k_\chi V_1}{2RT} \frac{v_m}{v_p} \Phi_e \cdot H^2 = 0 \qquad [5.25]$$

Numerical solution of the above equation provides the equilibrium concentration as a function of magnetic field intensity. This is shown in Fig. 5.21. Not only the equilibrium volume fraction, Φ_e, but also the swelling degree (defined as $q_V = 1/\Phi_e$) are shown in the figure.

On the basis of these figures it can be concluded that a significant effect of magnetic field on the equilibrium swelling degree can be expected at high field intensities. At small field intensities $\left(0 \le B \le 300 \text{ mT}\right)$ the change in the equilibrium swelling degree is comparable to the experimental accuracy. As the field intensity increases $\left(B \ge 300 \text{ mT}\right)$, significant decrease of the swelling degree is expected. Swelling experiments have shown that in the range $\left(0 \le B \le 300 \text{ mT}\right)$, no volume change was detected. Until now no



5.20 The influence of magnetic field on the chemical potential. The magnetic field strength varies from left to right as $10^{-5} \cdot H$ = 0, 5, 10 and 15 A/m.



5.21 The influence of magnetic induction on (a) the equilibrium volume fraction, as well as (b) the equilibrium swelling degree of the ferrogel.

experimental research has been reported on the dependence on the uniform magnetic field of equilibrium swelling degree.

5.7 Polymer gels in a non-uniform electric or magnetic field

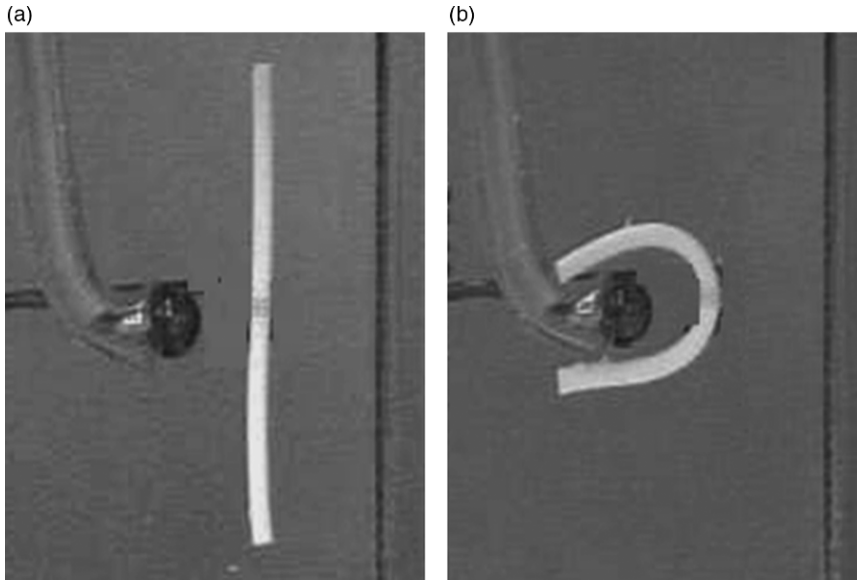
Electrorheological fluids, magnetorheological fluids and ferrofluids contain dispersed small particles in the size range from nanometres to micrometres (Jones, 1995; Tao and Roy, 1994) These fluids respond to an applied field by rapidly changing their apparent viscosity and yield stress. Since polymer gels contain substantial amounts of liquid as swelling agent, it is possible to fabricate field sensitive gels by using a polymer network swollen by a complex fluid. The incorporated colloidal particles, characterized by strong adsorptive interactions between solid particles and polymer chains, couple the shape and physical properties of the gel to the external field. These field sensitive gels can be exploited to construct new types of soft actuators, sensors, micromachines, biomimetic energy-transducing devices and controlled delivery systems.

If a field sensitive gel is exposed to an external field, two distinct types of interactions can be identified: *field–particle* interaction, as well as *particle–particle* interaction. If the field is non-uniform, then the field–particle interactions are dominant. Particles experience a dielectrophoretic (DEP), or magnetophoretic (MAP) force, respectively. As a result the particles are attracted to regions of stronger field intensities. Because of the cross-linking bridges in the network, changes in molecular conformation due to either DEP or MAP forces can accumulate and lead to macroscopic shape changes and/or motion. The main features of the DEP and MAP forces are summarized in Table 5.1.

Table 5.1 Particle–field interactions in non-uniform fields

Electric field	Magnetic field
Dielectrophoretic force (DEP) $f_{DEP} = 2\pi\varepsilon_1 R^3 K \nabla E_0^2$	Magnetophoretic force (MAP) $f_{MAP} = 2\pi\mu_1 R^3 K \nabla H_0^2$
Permittivities	*Permeabilities*
$K = \dfrac{\varepsilon_2 - \varepsilon_1}{\varepsilon_2 + 2\varepsilon_1}$	$K = \dfrac{\mu_2 - \mu_1}{\mu_2 + 2\mu_1}$
Field gradient ∇E_0	*Field gradient* ∇H_0
Low energy consumption Dangerous	Significant energy consumption Safe

Note: R = radius of solid particles, ε and μ = respective permittivity and permeability; the index 2 refers to the colloidal particle; the index 1 denotes the swelling agent.



5.22 Bending of a TiO_2-loaded PDMS gel in a non-uniform electric field. The TiO_2 content of this gel is 10 wt%: (a) no external electric field; (b) bending in non-uniform electric field.

The field sensitive gels can be made to repeatedly bend and straighten, as well as elongate and contract many times without damaging the gel (Feher *et al.*, 2001; Zrinyi, *et al.*, 2001, 2002). The response time to obtain the new equilibrium shape was found to be less than a second and seems to be independent of the size of the gel. This is demonstrated for magnetic field sensitive as well as electric field responsive gels in Figs 5.15 and 5.22. This latter figure shows that TiO_2 -loaded PDMS gel cylinder, suspended into silicon oil, undergoes significant bending deformation when an external electric field is applied. One of the electrodes is a metal ball with a diameter of 5.5 mm, the other electrode is a copper plate. By applying a DC field, the gel cylinder bends toward the metal ball. It is obvious from the pictures that the non-uniform field induces bending of the gel. A large deflection has been observed due to the DEP forces. It is also important to mention that the bending is rapid and the final equilibrium shape is reached within 5 s depending on the viscosity of the silicon oil and the size of the gel.

5.8 Future trends

The ability of magnetic field sensitive gels to undergo a rapid, controllable change of shape can be used to create an artificially designed system

possessing sensor and actuator functions internally in the gel itself. The peculiar magneto-elastic properties may be used to create a wide range of motion and to control the smooth and gentle shape change and movements that are similar to those observed in muscle. Thus, application of magnetic field sensitive gels in a soft actuator role for robots and other devices has special interest. Unlike in metallic machine systems, devices made of gels work without noise, heat evolution and exhaustion. An understanding of magneto-elastic coupling in gels will hasten gel engineering to include switches, sensors, micromachines, biomimetic energy-transducing devices and controlled delivery systems. If the magnetic field is created inside the gel by incorporating small powerful electromagnets and the field is coordinated and controlled by a computer, then the magnetic field sensitive gel may be used as an artificial muscle.

5.9 Acknowledgements

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5.10 References

Abramchuk S, Kramarenko E, Stepanov G, Nikitin LV, Filipcsei G, Khokhlov AR and Zrinyi M, (2007a) *Polym. Adv. Technol.* **18**(11):883–890.

Abramchuk S, Kramarenko E, Stepanov G, Nikitin LV, Filipcsei G, Khokhlov AR and Zrinyi M, (2007b) *Polym. Adv. Technol.* **18**(7):513–518.

Bacri J C and Salin D (1982) *J. Phys. Lett*. **43**, L179.

Bacri J C and Salin D (1983) *J. Phys. Lett*. **44**, 415.

Barsi L and Zrinyi M (1998) *ACH-Models Chem.* **153**(3):241.

Barsi L, Büki A, Szabó D and Zrinyi M (1996) *Progr. Colloid Polym. Sci*. **102**:57–63.

Berkovski B and Bashtovoy V (eds) (1996) *Magnetic Fluids and Applications Handbook*, Begell House, Inc.: New York, Wallingford.

Bajpai AK and Gupta R (2011) *J. Mater. Sci*., **22**:357–369.

Bossis G, Coquelle E and Kuzhir P (2004) *Ann. Chim. Sci. Mat.* **29**:43.

Carlson JD and Jolly MR (2000) *Mechatronics* **10**:555.

Choubey J and Bajpai AK (2010) *J. Mater. Sci: Mater Med*. **21**:1573–1568.

De Rossi K, Osada KY and Yamauchi A (eds) (1991) *Polymer Gels: Fundamentals and Biomedical Applications*, Plenum Press: New York.

Dormann JL and Fiorani D (eds) (1992) *Magnetic Properties of Fine Particles*, North-Holland: Amsterdam.

Dusek K and Prins W (1969) *Adv. Polym. Sci.* **6**:1.

Fehér GF, Szalma J and Zrínyi M (2001) *Colloid Surface, A: Physicochem. Eng. Aspects*, **183–185**:505–515.

Filipcsei G and Zrinyi M (2009) *Period Polytech-Chem Eng* Volume: 53 Issue: 2, 93–96.

Filipcsei G and Zrínyi M (2010) *J. Phys.: Condens. Matter* **22**:276001.

Filipcsei G, Csetneki I, Szilágyi A and Zrínyi M (2007) Magnetic field-responsive smart polymer composites (review), In: *Advances in Polymer Science, Oligomers, Polymer Composites, Molecular Imprinting*, Ed. Springer-Verlag: Berlin Heidelberg, pp. 137–189.

Ghost S and Cai T (2010) *J. Phys. D*. **43**:1–10.

Ginder JM and Davis LC (1994) *Appl. Phys. Lett.* **65**(26):3410.

Ginder JM, Clark SM, Schlotter WF and Nichols E (2002) *Int. J. Modern Phys. B* **16**:17:18, 2412.

Ginder JM, Nichols ME, Elie LD and Tardiff JL (1999) *Proc. SPIE* **3675**:131.

Gollwitzer C, Turanov A, Krekhova M, Lattermann G, Rehberg I and Richter R (2008) *J. Chem. Phys*. **128**(16):164709.

Hadijipanayis GC and Siegel RW (eds) (1994) *Nanophase Materials*, Kluwer Academic Publishers: Dordrecht, the Netherlands.

Jolly MR, Carlson JD, Munoz BC and Bullions TA (1996) *J. Int. Mater Syst. Struct.* **7**:613.

Jones TB (1995) *Electromechanics of Particles,* Cambridge University Press: New York.

Kilian H-G (1987) *Colloid Polym. Sci.* **265**:410.

Lien YH and Wu TM (2000) *J. Colloid Interface Sci.* **326**(2):517–521 Published: 15 October 2000.

Mark JE and Erman B (1988) *Rubber-like Elasticity A Molecular Primer*, John Wiley Sons: New York, Chichester, Brisbane, Toronto, Singapore.

Miller JS and Epstein AJ (1998) *Chem. Commun.* 1319.

Mitsumata T and Ohori S (2011) *Polym. Chem.* **2**:1063–1067.

Mitsumata T, Ikeda K, Gong JP, Osada Y, Szabó D and Zrinyi M (1999) *J. Appl. Phys.*, **85**(12): 1–5.

Nakano M and Koyama K (1997) *Electro-Rheological Fluids, Magneto-Rheological Suspensions and Their Applications*, World Scientific Publishing Co. Pvt Ltd: Singapore, New Jersey, London, Hong Kong.

Nobuhiko (ed.) (2004) *Reflexive Polymers and Hydrogels: Understanding and Designing Fast Responsive Polymeric Systems*, CRC Press: Boca Raton, London, New York, Washington D.C.

Okano T. (ed.) (1998) *Biorelated Polymers and Gels*, Academic Press: Boston, San Diego, New York, London, Sydney, Tokyo and Toronto.

Osada/Khokhlov (2002), *Polymer Gels and Networks*, Marcel Dekker Inc.: NY, Chapter 11, 309–355.

Patel R, Upadhyay RV and Mehta RV (2008) *J. Phys.: Condens. Matter* **20**:204116.

Restorf JB (1994) *Encyclopedia Appl. Phys.* **9**:229.

Rosenweig RE (1985) *Ferrohydrodynamics*. Cambridge University Press: Cambridge.

Raikher YL and Stolbov OV (2003) *J. Magn. Magn. Mater.* 258–259, 477.

Raikher YL and Stolbov OV (2005) *J. Magn. Magn. Mater.* **289**:62–65.

Sato O, Iyoda T, Fujishima A and Hashimoto K (1996) *Science* **272**:704.

Shiga T, Okada A and Karauchi T (1993) *Macromolecules* **26**:6958.

Shiga T, Okada A and Karauchi T (1995) *J. Appl. Polym. Sci.* **58**:787.

Szabó D, Barsi L, Büki A and Zrinyi M (1997) *Models Chem.* **134**(2):155.

Szabó D, Czako-Nagy I, Zrinyi M and Vertes A (2000) *J. Colloid Interface Sci.* **221**:166.

Szabó D, Szeghy G and Zrinyi M (1998) *Macromolecules* **31**:6541–6548.
Tanaka T (1982) *Science* **218**:467.
Tanaka T (1978) *Phys. Rev. Lett*. **40**:820.
Tao R and Roy GD (1994) *Electrorheological Fluids*, World Scientific Publishing Co. Pvt. Ltd: Singapore, New Jersey, London, Hong Kong.
Török Gy, Lebedev VT, Cser L and Zrinyi M (2000) *Physica B***276**:396.
Treloar LRG (1975) *The Physics of Rubber Elasticity*, Clarendon Press: Oxford.
Varga Z, Filipcsei G and Zrinyi M (2006) *Polymer* **47**(1):227–233.
Verdaguer M (1996) *Science* **272**:698.
Wood DS and Camp PJ (2011) *Phys. Rev. E* **83**, 011402.
Xulu M, Filipcsei G and Zrinyi M (2000) *Macromolecules*, **33**(5):1716–1719.
Zrinyi M (1997) *Trends Polym. Sci.* **5**:280.
Zrinyi M (2000) *Colloid Polym. Sci.* **27**(2):98.
Zrinyi M, Barsi L and Büki A (1996) *J. Chem. Phys.* **104**(20):8750.
Zrinyi M, Barsi L and BükiA (1997a) *Polym. Gels N.* **5**:415.
Zrinyi M. Barsi L, Szabó D and Kilian HG (1997b) *J. Chem. Phys.* **108**(13):5685.
Zrínyi M, Fehér J and Filipcsei G (2000) *Macromolecules* **33**(16) 5751–5753.
Zrinyi S, Szabó, D, Filipcsei G and Fehér J (2001) Electric and magnetic field-sensitive smart polymer gels, In: Y Osada and Khokhlov (eds), *Polymer Gels and Networks*, Marcel Dekker, Inc.: New York, Chapter 11, pp. 309–355.
Zrinyi M, Szabo D and Barsi L (1998) *J. Intell. Mater. Sys. Struct.* **9**:667.
Zrinyi M, Szabó D and Barsi L (1999c) In: Osada Y, Rossi DE (eds) *Magnetic Field Sensitive Polymeric Actuators. Polymer Sensors and Actuators*. Springer: Berlin, Heidelberg, New York, p 385 .
Zrinyi M, Szabó D and Fehér J (1999b) Electroactive polymer actuators and devices (ed.) Yoseph Bar Cohen, SPIE **3669**:406–413.
Zrinyi M, Szabó D, Filipcsei G and Fehér J (2002) In: Osada Y, Khokhlov A, Dekker M (eds) Electric and magnetic field sensitive smart polymer gels. Polymer gels and networks. CHIPS: New York, p. 309.
Zrinyi M, Szabo D and Kilian HG (1999a) *Polym. Gel N.* **6**(6):441.

6

Enzyme-responsive polymers: properties, synthesis and applications

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Abstract: Enzyme-responsive polymers are unique in their ability to interact with biological surroundings through mechanisms that enable dynamic responses by signal amplification under constant conditions. Research into enzyme-responsive polymers is gaining more impetus that will see the development of increasingly sophisticated systems and their integration in biomedical applications. In this chapter, we will first introduce the characteristics of enzyme-responsive materials in general before introducing the different types of enzyme-responsive polymers developed so far. We will then discuss the various strategies employed to introduce enzyme-responsive functionalities into the polymers. Finally, applications of enzyme-responsive polymers will be described.

Key words: enzyme-responsive materials (ERMs), regenerative medicine and drug delivery applications, polymer hydrogels and scaffolds, supramolecular particles and self-assembly polymer particles.

6.1 Introduction

Enzyme-responsive polymers stand apart from other stimuli-responsive polymers in their ability to respond to a biological molecule to regulate the function of natural materials. This is both a strength and a limitation: while enzyme-responsive polymers are uniquely suited to perform tasks in their niche area (i.e., in a biological surrounding), they cannot readily be used in other applications that do not preserve the activity of enzymes.

Within their designed areas of application, however, the versatility of enzyme-responsive polymers is unmatched by any other stimuli-responsive materials. Enzyme-responsive polymers have found applications as cell supports, injectable scaffolds and drug delivery systems and have been integrated with other stimuli-responsive polymers to obtain materials with closely tailored stimuli-responsive characteristics. While research in the development of enzyme-responsive materials (ERMs) is still in its early

stages, such materials are likely to play a major part in the effort to develop smarter biomaterials that are able to adapt to the demands of their biological surrounding. Since no biological system exactly equals another, adaptive stimuli-responsive materials that not only react to but interact with biological cues such as enzymes to provide a tailored response will become essential in future life science technologies.

6.2 Enzyme-responsive materials: rationale, definition and history

Enzymes are a relatively new class of stimuli for responsive materials. They have recently evolved from the realisation that biological processes can be harnessed to directly alter the properties of a material. This section will introduce the rationale behind using enzymes as stimuli, explain what enzyme-responsive materials are and explore their historical development.

6.2.1 Rationale behind enzyme-responsive materials

The use of physical or chemical stimuli (temperature, pH, light, small biomolecules) to alter the properties of a material is attractive because the stimulus can be well controlled and easily measured. Polymers have been widely used as materials that can be designed to respond to such stimuli in a predictable manner (Stuart *et al*., 2010). Application of the above stimuli typically occurs externally, providing good control over the material properties. The increasingly tighter connection of chemical, physical and engineering sciences with biological disciplines has introduced stimuli-responsive materials into the life sciences. Research on stimuli-responsive biomaterials aims to integrate the material and its function into a biological surrounding, that is, interface artificial materials with living organisms, and has applications in drug delivery, regenerative medicine, cell engineering and biomedicine (Alarcon *et al*., 2005; Cole *et al*., 2009; Sun *et al*., 2011). The complexity of natural organisms, however, far surpasses that of any artificial system, making both the application of a stimulus and the interaction of the material with its surroundings much more complicated. Thus, the requirements for a functional material in a biological setting have to be reconsidered. The external application of a stimulus may not be feasible when the material is inaccessible or if the stimulus is incompatible with the biological environment. Moreover, commonly used stimuli such as pH or temperature changes are not specific or targeted to the material but may affect other components of the environment as well.

In nature, dynamic processes in living organisms are almost exclusively controlled by enzymes. Enzymes have evolved to be highly specific catalysts

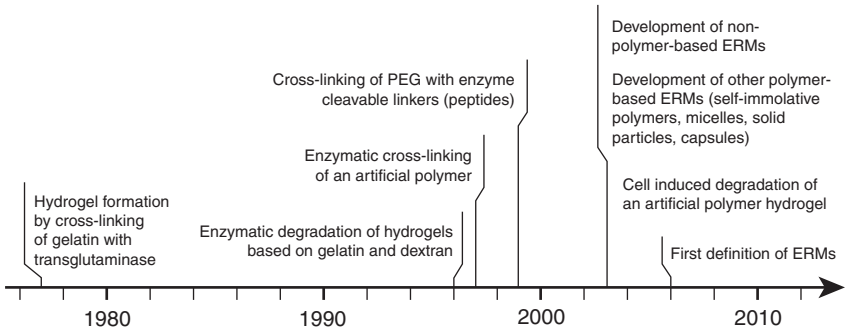
whose action and activity is naturally controlled either by regulating enzyme expression levels or the availability of cofactors. The reactions catalysed by enzymes are typically equilibrium driven and can therefore – in principle – be reversed. Enzymes thus present a highly attractive alternative to conventional stimuli for the development of functional biomaterials. For biological applications, enzymatic stimuli have further advantages. As they are already present in the biological environment, they are able to operate under the required conditions and do not have to be added externally (Ulijn, 2006). Moreover, the activity of certain enzymes is often linked to specific locations in the body and/or specific conditions of the organism. For example, several diseases are accompanied by a high level of phosphatase activity (Whyte, 2010), azoreductases can be found mainly in the digestive system (Miyata *et al*., 2002) and the healing of wounds displays a complex sequence of enzyme activities that mark the different stages in the wound healing process (Metcalfe and Ferguson, 2008). The incorporation of enzyme responsiveness into a material therefore goes beyond the addition of another stimulus to the repertoire; it presents the key to more closely integrating artificial materials with biological entities.

6.2.2 Definition of enzyme-responsive materials

The first definition of ERMs was presented in 2006 by R. V. Ulijn. In this work, ERMs were described as 'materials that undergo reversible macroscopic transitions that are triggered by selective enzyme catalysis' (Ulijn, 2006). Over the last decade, however, several materials have been designed that respond to an enzymatic stimulus but do not undergo a 'macroscopic transition' (e.g., enzyme-responsive self-immolative polymers, surfaces or quantum dots). The definition was therefore broadened subsequently, and Zelzer *et al*. (2013) defined ERMs as follows: 'The definition we use for ERMs encompasses materials whose structure or functionality changes after direct action of the enzyme. This excludes materials where enzymes are simply immobilised as well as systems where enzymes are merely employed to create a material (i.e., give the material its final form and function but otherwise leave the material inert to further enzymatic action) unless the enzymatic formation of the material in itself performs a function such as the formation of a hydrogel.'

6.2.3 Historical evolution

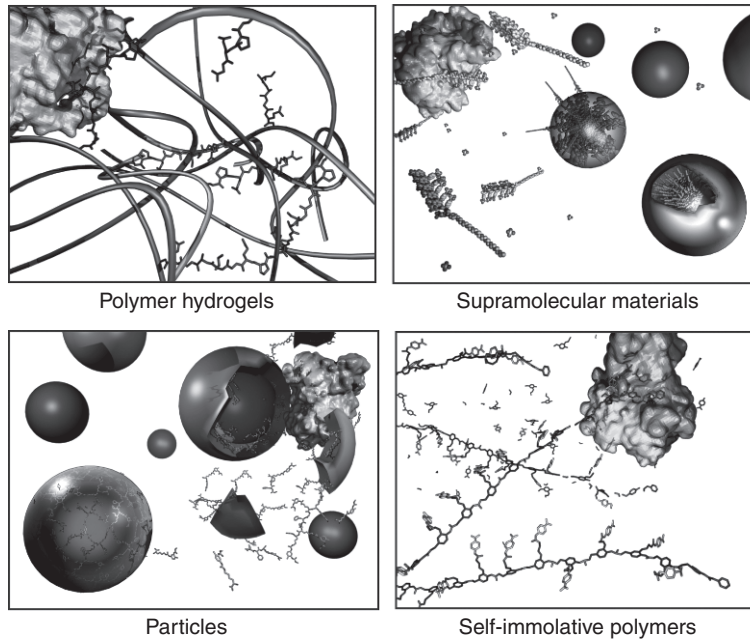
Compared to other stimuli-responsive materials, ERMs are a relatively young class of materials. An overview of the evolution of their research



6.1 Milestones in the development of ERMs.

is given in Fig. 6.1. Although first reports about the use of enzymes to alter the properties of a material date back as far as the 1970s (Folk and Finlayson, 1977; Fuchsbauer *et al*., 1996), where transglutaminase was used to cross-link gelatin (a naturally derived polypeptide), research on ERMs began to establish itself as a major discipline only at the end of the last century. Apart from the transglutaminase example above, the early focus of ERM research was on the enzymatic degradation of naturally derived materials such as gelatin and dextran hydrogels (Kurisawa *et al*., 1997; Yamamoto *et al*., 1996). Naturally derived polymers were therefore among the first ERMs developed. Not long after, artificial polymers were explored as ERMs: transglutaminase was used to cross-link glutaminamide functionalised poly(ethylene glycol) (PEG) with poly(lysine: phenylalanine) (Sperinde and Griffith, 1997) and methods were developed to cross-link polymers with short peptide sequences as enzyme-sensitive functionalities (West and Hubbell, 1999). The incorporation of short peptide cross-links into a polymer hydrogel was significant as the polymer was not required to be enzyme responsive in itself; instead the enzyme-sensitive functionality was introduced separately into the material, opening up the potential to use a variety of different enzymes to degrade the polymer hydrogel.

The next major breakthrough for ERMs came in 2003, when Hubbell and co-workers demonstrated that native enzymes provided by skin cells are able to degrade an artificial polymer hydrogel (Lutolf *et al*., 2003b). Now, ERMs do not merely present a different way of controlling material properties; they offer the possibility of linking material properties with biological processes in living organisms. Subsequently, research on ERMs has intensified significantly. ERMs expanded from polymer-based materials to include supramolecular materials (Yang *et al*., 2004), surfaces (Rawsterne *et al*., 2006; Yeo and Mrksich, 2003) and a variety of particles (Kanaras *et al*., 2003; Thornton *et al*., 2005; Zhao *et al*., 2003). Research on polymeric ERMs



6.2 Four classes of enzyme-responsive polymers. (*Source*: Reproduced in part from (Zelzer *et al.,* 2013).)

also diversified, expanding from polymer hydrogel based materials to polymer micelles, capsules and particles (Amir *et al*., 2009; Azagarsamy *et al*., 2009; Glangchai *et al*., 2008; Itoh *et al*., 2006), polymer conjugates (Kühnle and Börner, 2009) and self-immolative polymers (Amir *et al*., 2003; de Groot *et al*., 2003; Szalai *et al*., 2003). Currently, enzyme-responsive polymers can be placed in one of four classes (see Fig. 6.2): enzyme-responsive polymer hydrogels; enzyme-responsive supramolecular polymers; enzyme-responsive particles; and enzyme-responsive self-immolative polymers.

These first ERMs were mostly designed to respond to one enzyme in a non-reversible manner (i.e., enzymatic degradation of the material or the formation of cross-links). In nature, however, enzymatically regulated processes are often reversible and dynamic (e.g., the remodelling of extracellular matrix (ECM) or the formation of a cytoskeleton inside a cell). Recent developments in the design of ERMs attempt to emulate this behaviour, trying to build reversibility and dynamic processes as well as responsiveness to more than one enzyme into the material. The following sections will highlight important elements in the development of each class of enzyme-responsive polymers with a particular emphasis on the most recent accomplishments that will underpin the evolution of these materials towards academic and industrial applications.

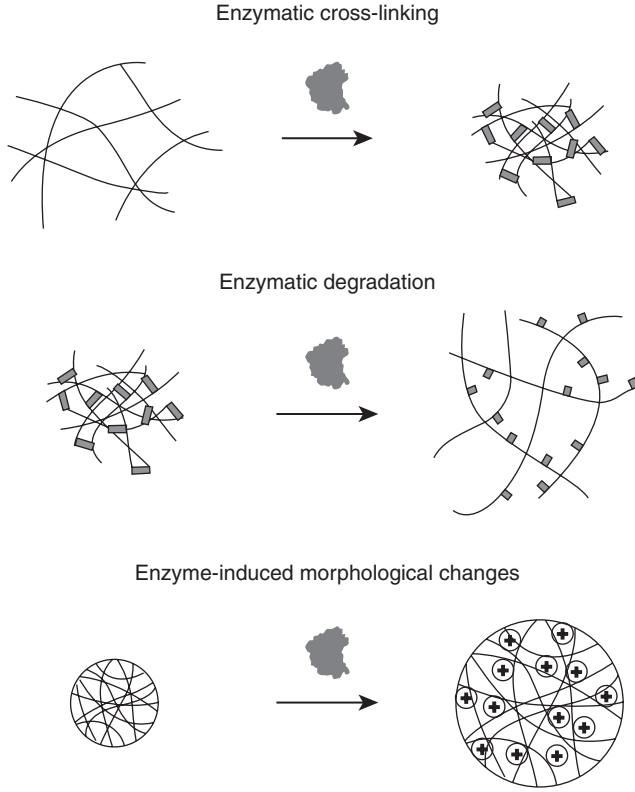
6.3 Key types and properties of enzyme-responsive polymers

Enzyme-responsive polymers can be classified in various ways, for example according to their structure, function or response type or mechanism. Here, we classify enzyme-responsive polymers, according to their structural elements, into polymer hydrogels, supramolecular polymers, polymer particles and self-immolative polymers. The general properties of these classes and their importance in biomaterial applications will be introduced. Using examples from the recent literature, we will demonstrate how enzyme responsiveness can be incorporated into these materials.

6.3.1 Polymer hydrogels

Polymer hydrogels consist of a cross-linked network of hydrophilic polymers with a very large water content (up to 99%) (Wichterle and Lim, 1960). They are structurally similar to the ECM and have therefore frequently been used as ECM mimics (Fedorovich *et al*., 2007; Shoichet, 2010). Other biological applications include the use as injectable scaffolds, (temporary) cell culture supports and drug delivery matrices (Mano, 2008). For all these applications, the introduction of enzyme-responsive functionalities into the polymer hydrogel is attractive because it would either more closely mimic the ECM (which is itself enzyme responsive) or allow drug delivery in response to the presence of a specific enzyme.

Polymers used for enzyme-responsive hydrogels include both naturally derived (e.g., polypeptides and polysaccharides) and artificial polymers (e.g., PEG/poly(ethylene oxide) (PEO), poly(2-hydroxyethyl methacrylate) and poly(N-isopropylacrylamide)). The first enzyme-responsive polymer hydrogels were designed to either form or degrade in the presence of an enzyme (Fig. 6.3). Hydrogel formation was accomplished either by enzymatically forming cross-links between the polymer strands (transglutaminase) (Fuchsbauer *et al*., 1996; Sperinde and Griffith, 1997) or by generating reactive groups that form cross-links with neighbouring strands (tyrosinase and horseradish peroxidase (HRP)) (Chen *et al*., 2002; Kurisawa *et al*., 2005). Polymer hydrogels based on natural polymers can often be degraded directly (e.g., cross-linked dextran by dextranase). Artificial polymers require the incorporation of enzyme-sensitive moieties, typically in the form of enzyme cleavable cross-linkers such as short peptide sequences. In this manner, polymer hydrogels that can be degraded with matrix metalloproteinases, elastase and other enzymes were prepared (Kim and Healy, 2003; Lutolf *et al*., 2003a; Mann *et al*., 2001; van Dijk *et al*., 2010; Yang *et al*., 2010). The use of peptides as cross-linkers has the advantage that the peptide sequence can be matched to the target enzyme of choice, whereas the response of naturally



6.3 Types of enzyme-responsive polymer hydrogels.

degradable polymers is restricted to one enzyme only. More control over the degradation of (naturally derived) polymer hydrogels can be achieved with dually responsive systems. In this instance, access of the enzyme to the enzyme-sensitive functionality is blocked until a second stimulus (pH, temperature, etc.) changes the structure of the hydrogels (Kumashiro *et al*., 2002; Sanborn *et al*., 2002). Equally, dually enzyme-responsive polymer hydrogel can be prepared. These materials can first be cross-linked with an enzyme to form the hydrogel, and then enzymatically degraded to destroy the hydrogel again (Ehrbar *et al*., 2007).

Enzymes can not only be used to create or destroy polymer hydrogels; they have also been employed to control the morphology of hydrogel particles (Fig. 6.3). By changing the overall charge of the particles, the degree of swelling can be changed using enzymes such as trypsin, thermolysin, elastase and matrix metalloproteinases (Thornton *et al*., 2005, 2008; McDonald *et al*., 2009; Patrick and Ulijn, 2010). A similar effect can be achieved by

reducing the number of cross-links within the hydrogel via enzymatic cleavage (Klinger *et al*., 2012).

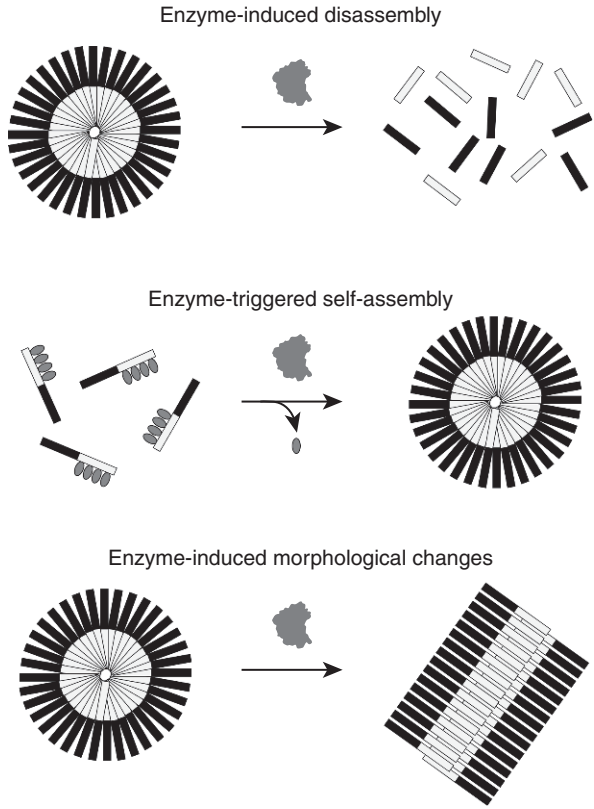
6.3.2 Supramolecular polymer structures

Supramolecular materials are architectures consisting of molecules that are able to self-assemble into larger constructs (Lehn, 2002). Polymers as well as small molecules are able to form self-assembled structures. Supramolecular materials based on small molecules such as peptides or peptide amphiphiles are also referred to as supramolecular polymers. Despite the importance of enzyme controlled self-assembly of small molecules, these materials will not be discussed here as they are not based on conventional polymers. More information on these materials can be found in the recent literature (Roy and Ulijn, 2010; Zelzer *et al*., 2013).

In nature, self-assembly is essential in many processes (see, for example, the cell membrane or the formation of cell adhesions), and frequently the assembly and disassembly of supramolecular materials is controlled enzymatically. The form and/or function of the final supramolecular material differs from that of the original building blocks, making them useful for applications such as drug delivery or regenerative medicine (Börner *et al*., 2010; Hahn and Gianneschi, 2011). The driving force behind the self-assembly of the building blocks can be based either on phase segregation due to hydrophilic/hydrophobic interactions (e.g., block polymers) (Amir *et al*., 2009) or on the formation of well-ordered secondary structures such as β-sheets through hydrogen-bonds (e.g., polypeptides) (Mutter *et al*., 2004). A combination of both mechanisms can be accomplished using conjugates of polymers with oligo- or polypeptides (Castelletto *et al*., 2010; de Graaf *et al*., 2012).

Similar to polymer hydrogels, enzymatic regulation of supramolecular materials falls into three categories (Fig. 6.4): formation of self-assembled structures, disassembly of supramolecular materials and control of the structures morphology (e.g., their shape or size). The main attraction of this class of materials, however, is their ability to undergo multiple and/or reversible transitions. Whether or not such transitions are possible depends on the design of the material, in particular on the way the enzymatic reaction is translated into a material response.

The use of amphiphilic block polymers to form micelles and other self-assembled structures is well known and documented (Riess, 2003). Enzymatically induced disassembly of such structures can be accomplished in two ways. Firstly, the hydrophilic and hydrophobic polymer blocks can be separated from each other through introduction of an enzyme-sensitive linker. To this end, polymer blocks joined by ester links (Azagarsamy *et al*.,



6.4 Types of enzyme-responsive supramolecular polymeric materials.

2009; Ge *et al*., 2011) and short peptide sequences (Castelletto *et al*., 2010; de Graaf *et al*., 2012) can be prepared that can be cleaved by esterases and proteases. Secondly, a double-hydrophilic block polymer can be used that is converted into a polymer amphiphile by masking the hydrophilicity of one of the polymer blocks. For example, this can be done with a PEG-poly(lysine) block polymer. In the presence of adenosine 5′-triphosphate (ATP), a so-called superamphiphile is formed, in which the poly(lysine) block is rendered hydrophobic due to the association of multiple ATP molecules to the block and the overall amphiphilic block polymer/ATP associate self-assembles into a micelle. Enzymatic digestion of ATP using a phosphatase recovers the hydrophilic nature of poly(lysine) and destroys the micelle (Wang *et al*., 2010).

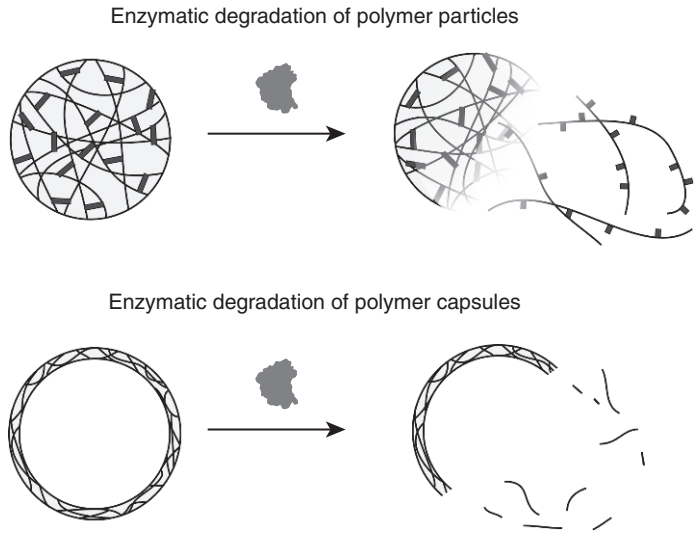
Enzyme mediated assembly of polymer-based supramolecular structures is mostly achieved by decreasing the hydrophilicity of one polymer block of a doubly hydrophilic block polymer. This can be done enzymatically by introducing phosphate groups to side chains of one block of the polymer.

Phosphatase-mediated dephosphorylation renders the block less hydrophilic and creates an amphiphile that is able to self-assemble (Amir *et al.*, 2009; Kühnle and Börner, 2009; Woodcock *et al.*, 2011). A second approach to enzymatically triggered self-assembly of polymers is restricted to materials that rely on the formation of well-ordered structures such as β-sheets and has currently only been shown for polypeptides. In these so-called 'switch peptides' defects are introduced into the peptide chain in the form of N-protected O/S-acyl isopeptides, and the hydrogen bonding pattern between the peptide chains is interrupted, preventing the formation of a secondary structure. Enzymatic removal of the N-protection group allows rearrangement of the peptide bond and removes the defect (for a detailed mechanism of this structural rearrangement see Fig. 6.12 in Section 6.4.2). This restores the hydrogen bonding pattern and causes self-assembly of the polypeptide into supramolecular structures (Mutter *et al.*, 2004).

The non-covalent nature of the interactions that drive the formation of supramolecular materials allows control of the material properties that goes beyond the enzyme-triggered assembly/disassembly of the structure. Small modifications such as the removal of one phosphate group by a phosphatase from a single amino acid attached to a polymer can trigger morphological transitions from spherical aggregates to micelles (Caponi *et al.*, 2011). Similarly, enzymatic reactions that alter the geometry of the polymer by extending the polymer chain (e.g., with phosphorylase *b* or DNA polymerase) (Alemdaroglu *et al.*, 2008; Morimoto *et al.*, 2007) or reducing the length of side chains (e.g., with DNAzyme) (Chien *et al.*, 2010) are able to change the size and shape of the self-assembled structure. Notably, some of these systems are completely reversible and can undergo these transitions multiple times (Ku *et al.*, 2011).

6.3.3 Polymer particles

Enzyme-responsive particles are particularly attractive as drug delivery or bio-imaging devices. To this end they typically act as carriers for other molecules that can be released by the enzymatic action on the material (Delcea *et al.*, 2011; Ghadiali and Stevens, 2008). In this chapter, we have already encountered two types of vehicles: polymer hydrogel particles and polymer micelles. Here, the introduction to these structures will be completed by a discussion of polymer particles that do not belong to the hydrogel or supramolecular materials family and we will distinguish two types of particles. Firstly, 'solid' particles are defined as particles in which the whole structure is filled by the polymer and the cargo is located between the polymer strands (Fig. 6.5). While micelles often fall into this category, they have already been discussed in Section 6.3.2. Solid particles can also be obtained by the random aggregation of polymers into spherical



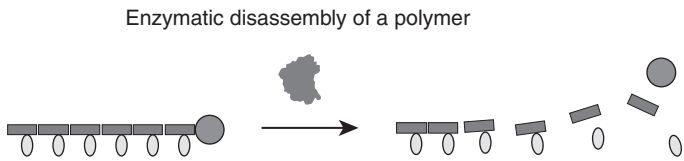
6.5 Types of enzyme-responsive polymer particles.

structures and by cross-linking polymers in a spherical shape, for example via emulsion-type polymerisations. Secondly, polymer capsules are materials where the polymer forms a solid cage around a hollow interior filled with solvent in which the cargo resides (Fig. 6.5). Notably, such capsules have not just been used to carry small drugs (Itoh *et al.*, 2006, 2008), they have also been employed to envelop and protect entire proteins (Biswas *et al.*, 2011; Gu *et al.*, 2009; Wen *et al.*, 2011). Enzyme responsiveness of these polymer particles and capsules is, however, restricted to degradation/disassembly of the polymer structure.

Solid particles that include peptides (e.g., polypeptide/artificial polymer conjugates) can be degraded directly with a protease (Habraken *et al.*, 2011). Particles based mainly on artificial polymers can be rendered enzymatically (proteolytically) degradable via the introduction of enzyme-sensitive cross-links (Glangchai *et al.*, 2008; Maier *et al.*, 2011). An exception to the protease-focused design of enzyme-responsive polymer particles is the use of phosphatase to change the electrostatic interaction of the polymer aggregates in the particle (Koga *et al.*, 2011). The enzyme-responsive polymer capsules designed so far exclusively rely on the direct degradation of the polymer, using either chitosanase or α-chymotrypsin (Itoh *et al.*, 2006, 2008).

6.3.4 Self-immolative polymers

Self-immolative polymers are carbamate or carbonate ester based polymers with specific architectures that allow 1–4 or 1–6 eliminations form metastable



6.6 Enzyme-responsive self-immolative polymers.

materials whose disassembly is prevented by a stable end-group on the polymer. Removal of this end-group causes a domino-like effect in which the polymer disassembles into its parental monomeric units. This is shown schematically in Fig. 6.6. If other molecules are attached to the polymer via the same metastable chemistry, these molecules will be released into the environment, making self-immolative polymers very useful carriers for drugs or marker molecules (Blencowe *et al.*, 2011).

Enzyme sensitivity is introduced to self-immolative polymers by designing end-groups for the polymer that can be enzymatically cleaved. Using this approach, dendrimer-like oligomers (Haba *et al.*, 2005; Shamis *et al.*, 2004), linear polymers (Weinstain *et al.*, 2009) and comb-polymers (Weinstain *et al.*, 2008) have been designed to disassemble in the presence of enzymes such as penicillin G amidase and catalytic antibody 28C2. This class of enzyme materials is still in its early stages but promises further potential in biomaterial applications (Zelzer *et al.*, 2013).

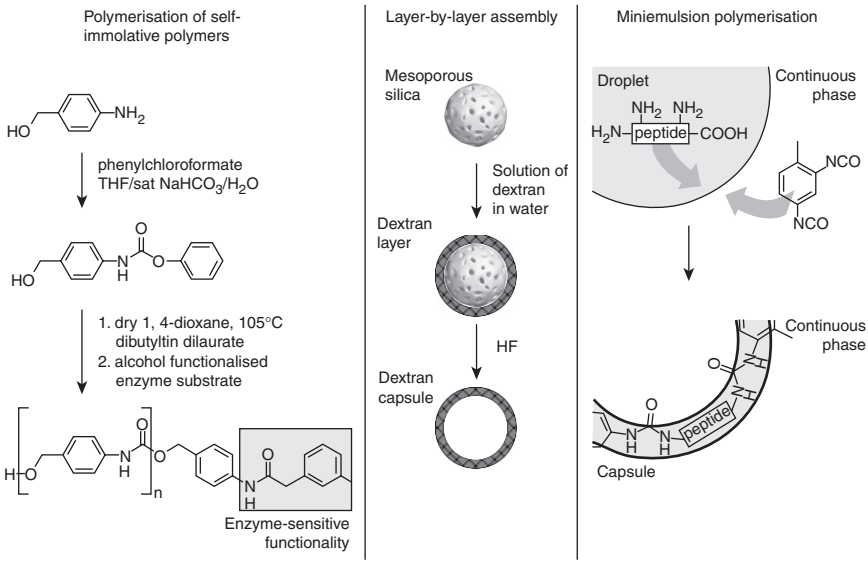
6.4 Preparation of enzyme-responsive polymers

While the previous section dealt with the structural elements that define enzyme-responsive polymers, here we will introduce different methods that can be used to integrate an enzyme-responsive functionality with a polymeric material. These methods are placed into three groups. The first method deals with the preparation of enzymatically degradable polymers, the second introduces strategies to incorporate enzyme-responsive linkers into the polymer and the third explores ways to prepare enzyme-responsive polymers enzymatically.

6.4.1 Enzymatically degradable polymers

Self-immolative polymers

The core of the self-immolative polymer structure is an amino-benzylalcohol that is connected via carbamate bonds. Reaction of the amine group with phenylchloroformate converts it into a phenylcarbamate which can be polymerised in a tin-catalysed reaction under exclusion of water. After



6.7 Fabrication methods for enzymatically degradable polymers.

allowing the polymerisation to proceed to completion, the end of the polymer chains are capped by addition of an alcohol. If the alcohol end-group is chosen such that it can be recognised as a substrate by an enzyme (see Fig. 6.7 for an example), exposure to the enzyme will remove the end-group and trigger the disassembly of the polymer (Weinstain *et al.*, 2008, 2009).

Layer-by-layer assembly

Hollow polymer capsules can be obtained by a template based process called layer-by-layer assembly. Mesoporous silica nanoparticles serve as temporary templates around which the polymer layers are constructed (Fig. 6.7). Immersion of the silica particles into an aqueous polymer solution, under addition of a cross-linking agent such as formic acid if required, results in the formation of a polymer layer around the particle. After washing, the process can be repeated with a different polymer to create the desired number of shells. Once the layer-by-layer assembly is complete, the mesoporous silica particle is removed by etching with hydrofluoric acid, generating a hollow polymer capsule. These capsules have been designed to be enzymatically degradable by using naturally derived polymers such as chitosan and dextran that can be digested by chitosanase and dextranase, respectively (Itoh *et al.*, 2006, 2008).

Miniemulsion polymerisation

Miniemulsion polymerisation processes are characterised by the presence of high shear forces and require both a surfactant and an osmotic pressure agent to stabilise the emulsion. Thus, suspensions of droplets with very small diameter (10–500 nm) and homogenous size distribution can be obtained, making it suitable for the preparation of nanoparticles (Schork *et al*., 2005). In addition, the polymerisation process in miniemulsions can be controlled such that polymerisation takes place preferentially at the interface of the two phases, allowing the formation of polymer capsules (Crespy and Landfester, 2010). In this a manner, oligopeptides with multiple amine functionalities (i.e., the peptide sequence contained several lysine units), were dissolved in the dispersed phase and cross-linked at the phase interface by addition of toluene diisocyanate (Fig. 6.7). The network density of the cross-linked polymer can be controlled by varying the amount of lysine units in the peptide sequence. The polymer capsule can be degraded enzymatically by proteolytic cleavage of the peptide (Andrieu *et al*., 2012).

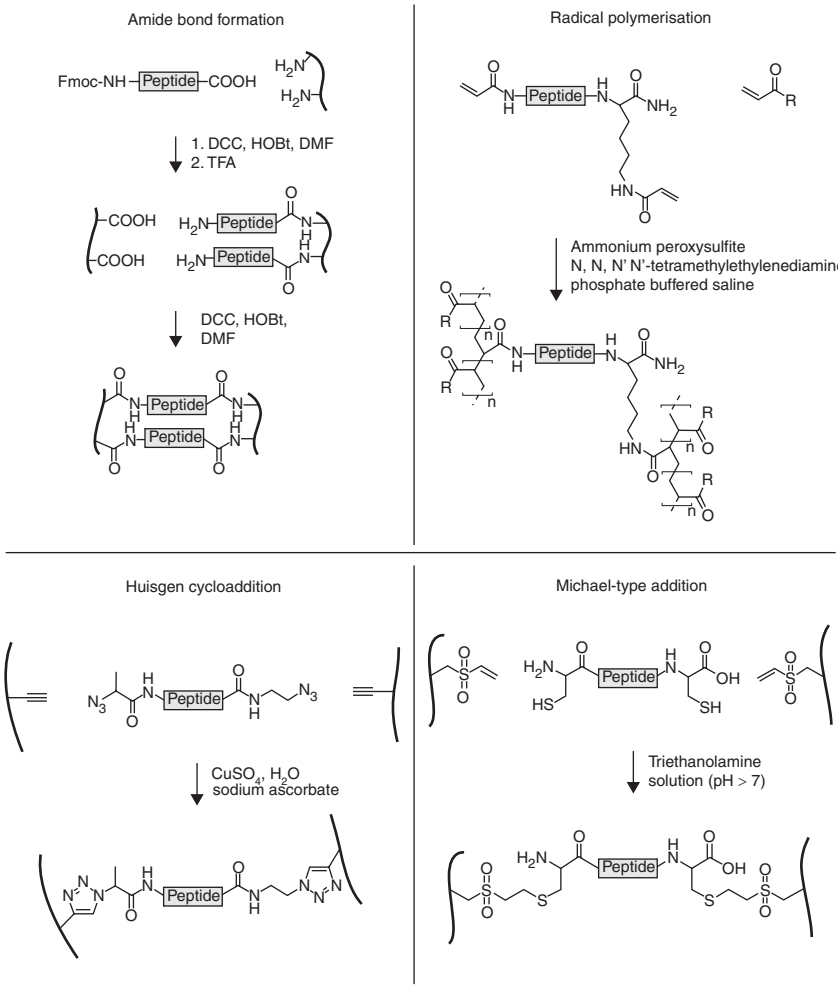
6.4.2 Introduction of enzyme-sensitive units

Cross-links

The introduction of enzyme-sensitive cross-links in an otherwise non-enzyme-responsive polymer such as PEG is frequently used to prepare enzyme-responsive polymer hydrogels and polymer particles. Because of their versatility and natural predisposition as enzyme substrates, short peptide sequences are almost exclusively used as cross-linkers, although dextran has also been used (Klinger *et al*., 2012). They can be readily changed to respond to a variety of proteases such as matrix metalloproteinases, plasmin or trypsin (Lutolf *et al*., 2003a; Yang *et al*., 2010). In most cases, the peptides have to be modified at the termini to introduce reactive groups that are able to react with the polymer.

There are four different chemical reactions that have been used to cross-link polymers with peptide sequences (Fig. 6.8): (i) amide bond formation; (ii) Michael-type addition; (iii) Huisgen cycloaddition (click reaction); and (iv) radical polymerisation. The amide bond formation follows typical solid phase peptide synthesis (SPPS) protocols and does not require functionalisation of the termini of the peptide sequence. Fluorenylmethoxycarbonyl (Fmoc) protection of the N-terminus allows attachment of the peptide sequence to an amine-bearing polymer. After removal of the Fmoc group, the amine-terminated peptide–polymer conjugate can be reacted with a second polymer bearing carboxylic acid functionalities using the same coupling chemistry (Maier *et al*., 2011). For Michael-type additions the peptide

termini do not have to be functionalised; instead the sequence has to be flanked by cysteine units. The thiol functionalities of the cysteine are able to react with vinyl sulfone units placed at the end of the polymer (e.g., a four-armed PEG) thus cross-linking the macromolecules (Lutolf and Hubbell, 2003). The incorporation of cross-links via a Huisgen cycloaddition requires modification of both the peptide and the polymer. Both azide- and alkyne-terminated peptides can be prepared and reacted with alkyne- and azide-functionalised polymers, respectively (van Dijk *et al.*, 2010; Yang *et al.*, 2010). Finally, peptides can be end-functionalised with polymerisable groups. These are typically acrylates that will be incorporated into the polymer *in situ*



6.8 Methods for the introduction of enzyme-sensitive cross-linkers.

during the formation of the polymer (Glangchai *et al.*, 2008; Gu *et al.*, 2009; Khelfallah *et al.*, 2006; Kim and Healy, 2003; West and Hubbell, 1999).

The mode in which the cross-linker is introduced into the polymer differs and is sometimes determined by the chemical reaction used. In the case of acrylated peptides, the polymer is not prepared in advance but formed in the presence of the cross-linker. When the unmodified peptide is attached to the polymer via amide bonds, the introduction of the linker has to be carried out in two stages. First, a peptide–polymer conjugate with an amine-functionalised polymer is formed, then the conjugate is reacted with a carboxylic acid-functionalised polymer. The other two methods – Huisgen cycloaddition and Michael-type addition – allow direct cross-linking of the polymer with the peptide.

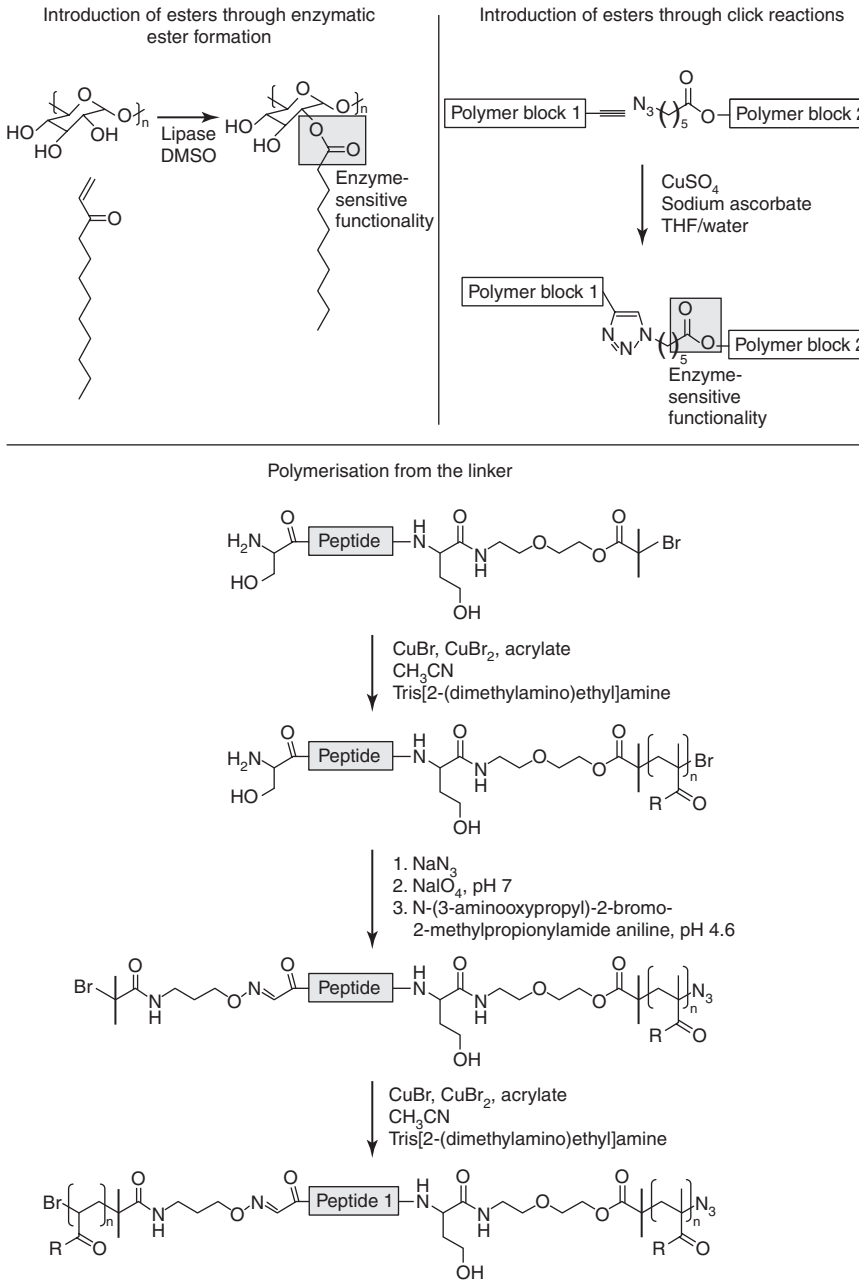
Linkers between polymer blocks

Self-assembling polymer amphiphiles are rendered enzyme responsive by introducing an enzymatically cleavable linker between two polymer blocks (Fig. 6.9). Esters can be readily used as linkers between the polymer blocks for esterase sensitive materials. In this case, the two polymer blocks were prepared separately with functional groups on one end. A lipase sensitive, dextran based polymer amphiphile was synthesised by enzymatic formation of an ester bond (Ge *et al.*, 2011). This is an example where lipase is used to both create and cleave the same bond. Alternatively, the ester can be prepared beforehand near the end of one of the polymer blocks, followed by an azide, allowing coupling to the second alkyne-terminated block via a Huisgen cycloaddition (Azagarsamy *et al.*, 2009; Raghupathi *et al.*, 2011).

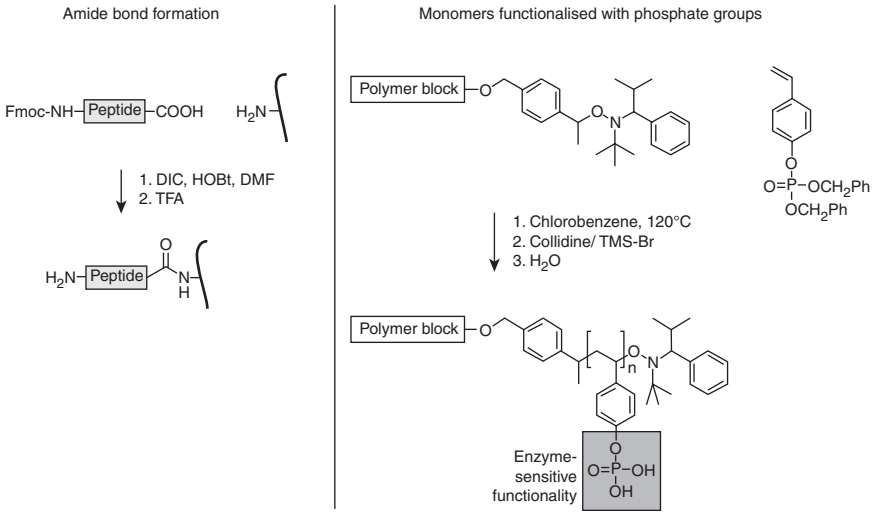
Instead of functionalising the polymer blocks after formation, the polymers can also be synthesised directly from the linker. A peptide sequence with the desired enzyme sensitivity can be modified on one end with an atom transfer radical polymerisation (ATRP) initiator and used to grow the first polymer block in a living polymerisation. Once complete, the second terminus of the peptide can be similarly modified and used to initiate the polymerisation of the second polymer block (de Graaf *et al.*, 2012).

Side chain functionalities

Incorporation of enzyme-sensitive units in the polymer side chain can be done either after the main polymer has been synthesised or the enzyme-sensitive functionality can be present in the monomer before polymerisation (Fig. 6.10). Attachment to the polymer is typically done via standard SPPS protocols. Both peptides and DNA fragments have been attached to polymers obtained through ring opening polymerisation of norbornene using either N-hydroxysuccinimide (NHS) activation or *N,N,N′,N′*-tetramethyl-



6.9 Methods for the introduction of enzyme cleavable linkers between polymer blocks.



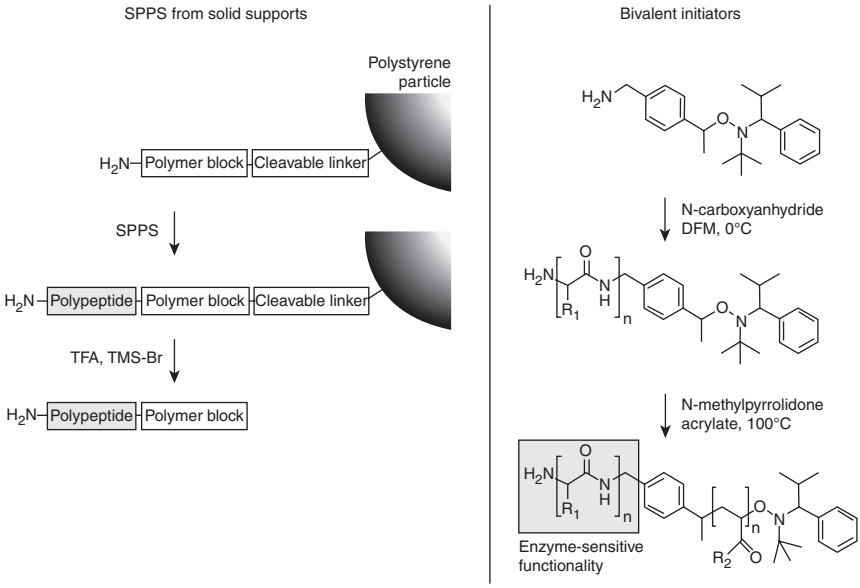
6.10 Methods for the introduction of enzyme-sensitive groups in polymer side chains. DIC, N,N′-diisopropylcarbodiimide; HOBt, 1-hydroxybenzotriazole; DMF, dimethylformamide; TFA, trifluoroacetic acid; TMS-Br, trimethylsilyl bromide.

O-(1*H*-benzotriazol-1-yl)uronium (HBTU) catalysed amide formation (Chien *et al.*, 2010; Ku *et al.*, 2011). Direct build-up of a peptide sequence from poly(ethylene glycol acrylamide) (PEGA) based hydrogel particles using carbodiimide/HBTU has also been demonstrated (Thornton *et al.*, 2005). The attached DNA/peptides convey enzyme-sensitive material response by altering either the electrostatic or self-assembly properties of the polymers after cleavage with an endonuclease or a protease.

Enzymatically cleavable phosphate groups have been introduced into the side chain of acrylate and styrene based polymers prepared via ATRP and nitrogen-mediated polymerisation, respectively. In both cases, the phosphate groups had to be protected during the polymerisation, using either *tert*-butyl or benzyl protection groups. After removal of these protection groups, the structural arrangement of these polymers could be changed by removing the phosphate groups with phosphatase (Amir *et al.*, 2009; Woodcock *et al.*, 2011).

Polymer conjugates

Enzyme-responsive polymer conjugates are typically block polymers of an artificial polymer and a polypeptide. Three strategies have been explored to prepare polymer–polypeptide conjugates. Firstly, the artificial polymer (e.g., PEG/PEO) can be immobilised on a solid support and terminated with an amine group (Fig. 6.11). This makes direct SPPS of the polypeptide

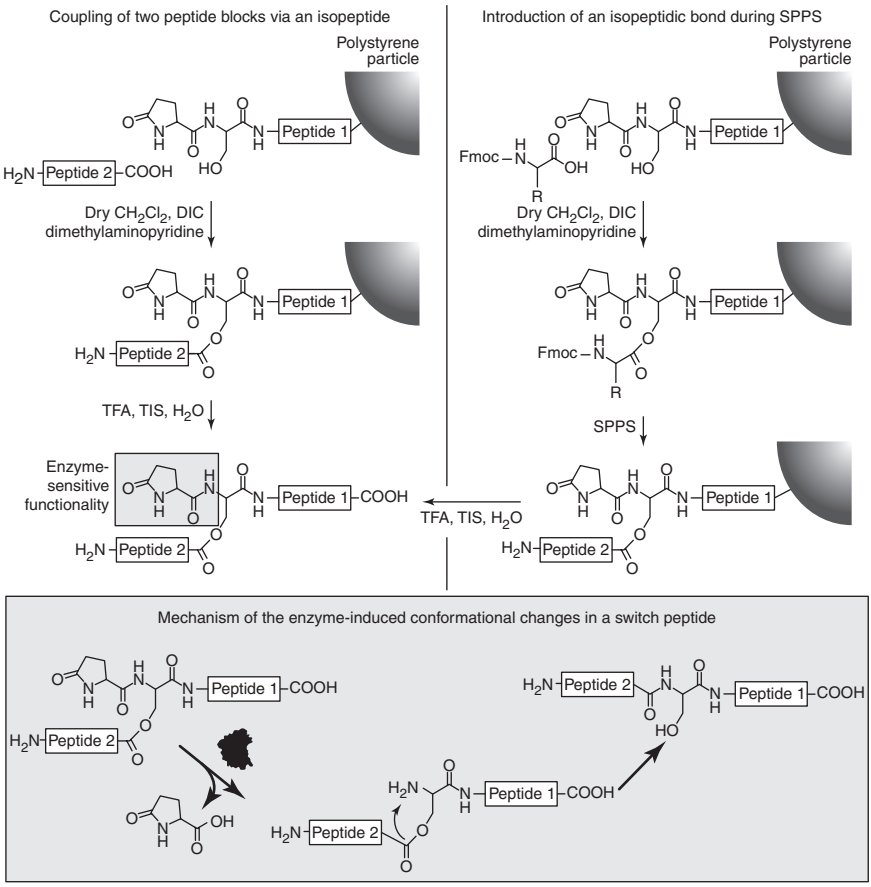


6.11 Methods for the preparation of enzyme-sensitive polymer conjugates.

from the artificial polymer possible (Castelletto *et al*., 2010; Kühnle and Börner, 2009). Secondly, the polypeptide and the artificial polymer can be synthesised sequentially from a bivalent initiator (Fig. 6.11). This initiator bears an amine group that allows the formation of a polypeptide via N-carboxyanhydride and a nitrogen-mediated polymerisation initiator for the formation of the artificial polymer (Habraken *et al*., 2011). Thirdly, a Huisgen cycloaddition can be used to attach amino acids to a polymer (see Fig. 6.9 for a similar example using the Huisgen cycloaddition based click chemistry to connect two polymer blocks). The alkyne can be incorporated in the initiator (e.g., for the ring opening polymerisation of 2-isopropyl-2-oxazoline)while the carboxy terminus of an Fmoc-protected amino acid can be modified with an azide functionalised amine with standard SPPS protocols (Caponi *et al*., 2011).

Switch peptides

The synthesis of the switch peptide follows standard SPPS protocols. Incorporation of the defect can be accomplished in two ways. The peptide strands on either side of the defect can be prepared separately (Fig. 6.12). The amino acid used to introduce the defect (serine, tyrosine or cysteine) has to be N-terminal to one peptide strand and remain N-protected with the enzymatically cleavable protection group. The C-terminus can then be



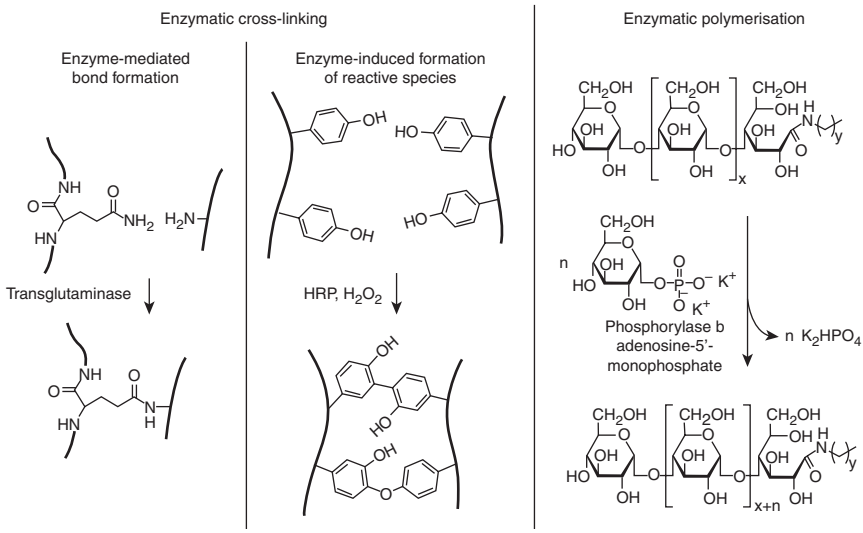
6.12 Methods for the preparation of enzyme-sensitive switch peptides. TIS, triisopropylsilane.

coupled to the alcohol/thiol to form the ester/thioester bond that will rearrange into an amide bond once the nitrogen protection group is removed. Alternatively, the defect can be built into the sequence directly during SPPS using the same reactions as above (Mutter *et al.*, 2004).

6.4.3 Enzymatic synthesis

Enzymatic cross-linking of polymers

The formation of cross-links relies on the ability of an enzyme to induce the formation of a covalent bond. Since most enzymes are very specific to their substrate the polymers have to be modified with specific functionalities to allow enzymatic cross-linking to take place. Transglutaminase is able to form



6.13 Methods for the enzymatic synthesis of polymers.

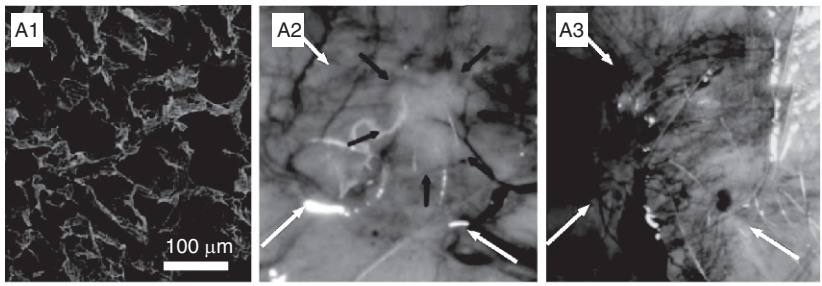
an amide bond between the α-carboxamide group of glutamine and a primary amine (Fig. 6.13). Naturally derived polymers such as gelatin contain a variety of different amino acids, including glutamine and lysine, which can be readily cross-linked with transglutaminase (Fuchsbauer *et al.*, 1996). This same reaction can be used to cross-link a conjugate of PEG and poly(lysine/phenylalanine) if the PEG molecules are end-functionalised with glutamine (Sperinde and Griffith, 1997).

Polymers can also be cross-linked if the enzyme generates reactive functionalities in the polymer chain (Fig. 6.14). Tyramine, for example, can be incorporated into the polymer (e.g., hyaluronic acid, dextran or alginate) and used to cross-link the material through oxidation of the phenol by HRP in the presence of H_2O_2 (Jin *et al.*, 2007; Kurisawa *et al.*, 2005; Sakai and Kawakami, 2007). In polypeptides, tyrosinase has also been used to oxidise tyrosine to quinone moieties that are able to react further with the functional groups in the side chains of a number of other amino acids (Chen *et al.*, 2002).

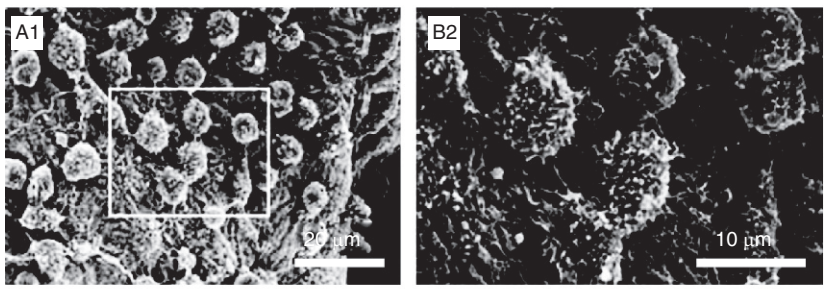
Enzymatic polymerisation

The extension of a polymer chain through enzymatic polymerisation results in a material response when the self-assembling properties of the polymer are changed as a result. Phosphorylase b has been used to extend the amylose part of a polymer amphiphile by attaching further o-glucose-1-phosphate units to the end of the amylose chain (Morimoto *et al.*, 2007) (Fig. 6.13). Terminal deoxynucleotidyltransferase (a DNA polymerase) was used to

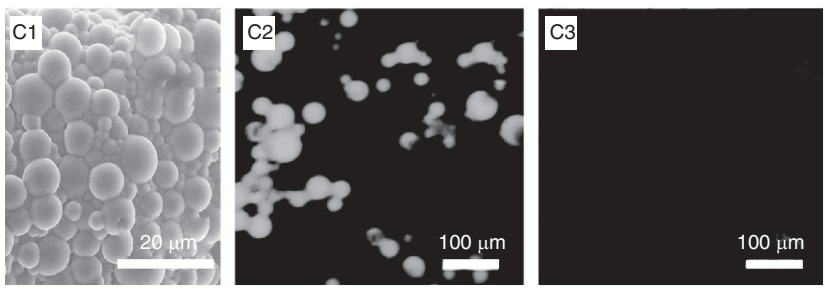
(a) Cell supports



(b) Injectable scaffolds



(c) Drug delivery



6.14 Applications of enzyme-responsive polymers. (a) Elastase sensitive polyurethane scaffold used as degradable cell supports. A1 shows SEM images of cross sections of the polymer hydrogel while A2 and A3 show photographs of subcutaneously implanted scaffolds (in rats) after 4 and 8 weeks, respectively (white arrows indicate the sutures and black arrows the edge of the hydrogel). (*Source*: Reproduced with kind permission from Springer Science and Business Media from (Guan *et al.*, 2008).) (b) Polymer hydrogels formed by enzymatically triggered gelation after injection. B1 and B2 show SEM images of chondrocytes incorporated *in situ* into a gel formed by the action of HRP/H_2O_2 on a hyaluronic acid/dextran conjugate. The white area in B1 is magnified in B2. (*Source*: Reprinted from (Jin *et al.*, 2010) with permission from Elsevier.) (c) Enzyme-responsive PEGA particles designed as drug delivery vehicles. C1 shows an ESEM image of the particles. C2 shows fluorescence microscopy images of the particles loaded with a fluorescently labelled payload and C3 shows the removal of the payload from the particles after exposure to thermolysin. (*Source*: Reproduced by permission of The Royal Society of Chemistry from (McDonald *et al.*, 2009).)

extend the oligonucleotide chain of a DNA-polypropylene oxide conjugate (Alemdaroglu *et al*., 2008).

6.5 Characterisation of enzyme-responsive polymers

Characterisation of the enzyme-responsive material prepared through the methods outlined above is essential not only to test the enzyme responsiveness of the polymer but also to characterise the material's overall performance under the conditions in which it will be used in its ultimate application. This section will provide a brief overview of standard and specialised techniques that have been employed for this purpose and covers mechanical, chemical, physical and biological properties as well as enzyme responsiveness. While we will discuss the reason for choosing particular techniques and explain their advantages and limitations in the context of the analysis of enzyme-responsive materials, explanations of the working principles of the techniques will not be provided and the reader is referred to other specialised textbooks on this topic instead.

6.5.1 Physical properties

Morphology

When the polymer is designed to possess a certain size/shape (particles) or if, indeed, the change of the size/shape of the material is the desired material response to the enzymatic action (supramolecular materials), accurate measurements of these properties are crucial. Imaging tools such as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM) are therefore essential tools to characterise these materials and do not differ from standard analysis tools of other stimuli-responsive materials (Chien *et al*., 2010; Ge *et al*., 2011; Itoh *et al*., 2006; Klinger *et al*., 2012; Ku *et al*., 2011; Kühnle and Börner, 2009). An important consideration for ERMs, however, is the fact that they have to be designed to operate under physiological conditions (i.e., aqueous environment, pH ~ 7.3, ~ 37°C). Measurement in the dry state (AFM) or under vacuum (SEM, TEM) often does not reflect the material properties under physiological conditions. Cryo-options are available for SEM and TEM to preserve the sample in a more natural state (Castelletto *et al*., 2010; Habraken *et al*., 2011; Wang *et al*., 2010; Yang *et al*., 2010;), whereas AFM measurements can be done directly in the liquid phase (Alemdaroglu *et al*., 2008). While these three techniques are suitable for objects with dimensions in the nanoscale, microscale-sized materials may also be imaged in liquid with bright field or fluorescence microscopy. An example of this is the use of two-photon spectroscopy to image microgel particles (McDonald *et al*., 2009; Thornton *et al*., 2005).

Non-imaging techniques can also provide information about the morphology or size of a particle in a liquid environment. Light scattering and X-ray diffraction techniques can also be used to obtain information about the shape and size of the material in an aqueous environment (Azagarsamy *et al*., 2009; Castelletto *et al*., 2010; Wang *et al*., 2010; Woodcock *et al*., 2011). Circular dichroism has been used to obtain information about the secondary structure of peptide-based ERMs (Kühnle and Börner, 2009; Mutter *et al*., 2004).

Mechanical properties

When the polymer material is used as a scaffold (e.g., polymer hydrogels), its mechanical properties become important because cells are known to respond to the stiffness of a substrate. Rheology is commonly used to characterise the mechanical properties of polymer hydrogels and can equally be used to characterise ERMs (Chen *et al*., 2002; Kim and Healy, 2003; Sanborn *et al*., 2002; van Dijk *et al*., 2010). In addition, to measure viscoelasticity on a micrometre scale, microrheology can be used. This method is able to measure local inhomogeneities in the material and is therefore able to measure inside small samples and cells (Yang *et al*., 2010).

6.5.2 Chemical properties

Chemical composition

The tools used to characterise the chemical composition of enzyme-responsive polymers are essentially the same as for other polymer systems and include NMR, IR- and UV/Vis spectroscopy, mass spectrometry, gel permeation chromatography (GPC) (Amir *et al*., 2009; Caponi *et al*., 2011; Chen *et al*., 2002; de Graaf *et al*., 2012; Ge *et al*., 2011; Habraken *et al*., 2011; Kim and Healy, 2003; Wang *et al*., 2010; Woodcock *et al*., 2011). Changes in a peptide sequence (i.e., cross-linking through amino acid side chains) are typically investigated by enzymatic digestion of the peptide and subsequent chromatographic analysis (Fuchsbauer *et al*., 1996). Special methods have been developed to follow some modifications. For example, the tyrosinase induced cross-link formation was followed by monitoring the consumption of oxygen needed during the bond formation (Chen *et al*., 2002).

6.5.3 Biological properties

Toxicity and inflammatory response

The toxicity and inflammatory response of the polymer are crucial characteristics of the enzyme-responsive material if it is to be used in a living

organism. The toxicity/inflammatory response of both the material and its degradation products need to be considered. The selection of the polymer is often steered towards materials that are already approved by governmental regulation agencies for biomedical applications (e.g., PEG) (Mann *et al*., 2001; Sanborn *et al*., 2002; Yang *et al*., 2010). Toxicity studies of new polymeric materials proposed as potential enzyme-responsive biomaterials, however, should be rigorously undertaken. Tests such as chloromethylfluorescein diacetate and live/dead assays on cells are available to monitor the viability of cells in the presence of the ERM (Ehrbar *et al*., 2007; Mann *et al*., 2001).

Metabolism and biodegradability

If the enzyme-responsive material performs a temporary function inside the body, pathways for the removal of the material need to be considered. Particles can often be metabolised and degraded in the blood stream, whereas hydrogels will be designed to slowly degrade locally over time. Degradation studies are typically performed by monitoring the weight loss gravimetrically and analysing the degradation products chromatographically (Kim and Healy, 2003; Kumashiro *et al*., 2002; van Dijk *et al*., 2010; Yang *et al*., 2010). For particles, light scattering and turbidity measurements have been used (Klinger *et al*., 2012).

6.5.4 Enzyme-responsive properties

Selectivity and cross-reactivity

Biological systems are extremely complex and contain a vast number of different enzymes. Many of these enzymes have evolved to catalyse very specific reactions, that is, they convert their substrate with high selectivity. Selectivity is the ability of an enzyme to convert one specific substrate in the presence of a mixture of molecules that are very similar to the target substrate. To perform correctly, ERMs must also be selectively recognised by the target enzyme. These properties are readily investigated by studying the behaviour of the ERM in the presence of other proteins. Selectivity of the enzyme to the enzyme-sensitive functionality is explored, for example, by slightly altering the peptide sequence and studying the response of the material to the enzyme (Ehrbar *et al*., 2007).

Another important consideration is the cross-reactivity of the enzyme-sensitive functionality with other enzymes. Ideally, the enzyme-sensitive functionality should react only with the enzyme that it was designed for and show little to no sensitivity to other enzymes. Similar to the selectivity, the cross-reactivity with other enzymes can be explored by exposing the

material to a selection of different enzymes that catalyse similar reactions (McDonald *et al*., 2009; Thornton *et al*., 2008).

Response characteristics

The response characteristics of the ERM are, of course, a vital property of the material. The response time of the material to the stimulus includes important parameters such as the lag time between enzyme reaction and material response, the effect of enzyme concentration on the material response and the duration of the material response. These properties are most frequently studied in systems that are designed for the delivery of drugs as the release kinetics of drugs are vital for treatment (McDonald *et al*., 2009; Itoh *et al*., 2006; Koga *et al*., 2011; Patrick and Ulijn, 2010; Wang *et al*., 2010; Weinstain *et al*., 2009). In many ERMs the response time is actually rather slow compared to other stimuli-responsive mechanisms and can take several hours to days to reach completion.

In reversible or dynamic ERMs, the demonstration of the repeated and reversible response of the material to the stimulus has to be characterised. This has been shown with two different enzymes that catalyse antagonistic reactions (phosphatase and kinase/ATP) (Ku *et al*., 2011).

6.6 Applications

By nature, ERMs are inherently suitable for applications in the healthcare section. Despite being a young class of materials, some exciting applications have begun to emerge. Here, three applications for enzyme-responsive polymers are highlighted: cell supports, injectable scaffolds and drug delivery devices.

6.6.1 Cell supports

With their high water content and three dimensional structure, polymer hydrogels possess characteristics that are very close to the environment in which many cells grow naturally. The hydrogel is strong enough to provide mechanical support for cells, while the flexible and highly hydrated 3D network facilitates the flux of nutrients to the cell and allows the cells to grow into 3D tissue. In nature, this environment is provided by the ECM, a hydrogel-like network of proteins (e.g., collagen from which gelatin is derived) and polysaccharides (e.g., hyaluronic acid) (Lutolf and Hubbell, 2005; Tibbitt and Anseth, 2009). An important characteristic of the ECM is its ability to be remodelled; that is, it can be broken down and reformed by enzymes such as matrix metalloproteinases. By introducing enzyme-sensitive crosslinkers into a polymer hydrogel, a close mimic of an ECM can be created

that can be degraded by enzymes expressed by the cells, allowing the cell to remodel its environment (Ehrbar *et al*., 2007; Guan *et al*., 2008; Lutolf *et al*., 2003a; Zisch *et al*., 2003). This ability makes enzyme-responsive polymer hydrogels powerful tools for the creation of new tissue in the lab and to support wound healing in a living organism. An example of such a cell-degradable hydrogel is shown in Fig. 6.14a. In this case, an elastase degradable polyurethane was prepared by pre-polymerising polycaprolactone diol with 1,4-diisocyanobutane and subsequently extending the polymer chain with an elastase cleavable diamine peptide (Ala-Ala-Lys). The porous scaffold shown in Fig. 6.14 A1 was obtained by thermally induced phase separation of the polyurethane. The scaffold was subsequently implanted into a rat where it was degraded by enzymes produced by the surrounding tissue, showing a significantly reduced size four weeks after implantation (Fig. 6.14 A2; white arrows indicate sutures, i.e., size of the original scaffold; black arrows indicate the edge of the reduced scaffold). Eight weeks after implantation, the scaffolds were fully degraded (Fig. 6.14 A3) (Guan *et al*., 2008).

Enzymatically degradable materials can also be combined with an enzymatically triggered release of growth factors to direct the response of the cell and gain a better control over the formation of the tissue (Phelps *et al*., 2010; Zisch *et al*., 2003). This is of particular importance for the engineering of blood vessels in the artificial tissue (Phelps *et al*., 2010). Enzymatically degradable hydrogels have also been used to generate cell sheets which are attractive for wound healing applications. After growing fibroblasts to a confluent sheet on the polymer hydrogel, the enzyme was added to degrade the polymer and release the cell sheet (Sakai *et al*., 2009b).

6.6.2 Injectable scaffolds

Injectable scaffolds are designed to change their physical properties after exposure to a stimulus, changing from a liquid to a self-supporting hydrogel. Placing materials of sufficient mechanical strength to support cell growth (such as a self-supporting hydrogel) into a body is difficult without surgical intervention (Jin *et al*., 2010). Due to the high viscosity of the hydrogel it cannot be injected through a needle. A material that is liquid during application and only gels after it has been placed into the body presents a solution to this problem. While several triggers can be used to induce hydrogelation of such an injectable scaffold, enzymes are particularly suited as they are already present in the body. Both supramolecular and polymer hydrogels have been used as enzyme-responsive injectable scaffold (Kurisawa *et al*., 2005; Yang *et al*., 2006). Supramolecular scaffolds are based on small peptide amphiphiles and therefore do not fall into the remit of this chapter. Injectable polymer hydrogels can be prepared from tyramine conjugates

with dextran or hyaluronic acid. A combination of these materials with HRP and H_2O_2 yields an injectable mixture that turns into a hydrogel in a matter of minutes. The gelation time can be varied by varying the amount of tyramine in the conjugate or by changing the enzyme concentration in the mixture (Jin *et al*., 2007, 2010; Kurisawa *et al*., 2005). HRP has also been used to induce gelation in a gelatin based injectable scaffold (Sakai *et al*., 2009a).Before injection of the material, cells can be added to the mixture for simultaneous delivery with the gel precursors to be incorporated into the gel during the enzymatic cross-linking. The SEM images in Fig. 6.14b show an example of such gel-encapsulated cells. Cell encapsulation was accomplished by combining a suspension of chondrocytes and the hydrogel precursor – a hyaluronic acid based polymer backbone carrying grafted side chains of dextran-tyramine conjugates – in cell culture media with a solution of HRP and H_2O_2 in phosphate buffered saline. The HRP cross-links the polymer through the tyramine moieties, trapping the cells inside the resulting hydrogel (Jin *et al*., 2010).

Enzyme responsiveness in injectable hydrogels has also been used to render the material biodegradable such that it can be removed once it is no longer required. In this case, poly(N-isopropylacrylamide-*co*-acrylic acid) that included peptide-based cross-links was used as the stimuli-responsive polymer. Hydrogelation was triggered thermally, whereas the enzyme-responsive functionality is used to provide proteolytically degradable sites in the hydrogel (Kim and Healy, 2003).

6.6.3 Drug delivery

Enzyme-triggered release of bioactive molecules from a polymer based material is possibly the most intensely researched application for enzyme-responsive polymers. Such delivery systems typically employ vehicles such as micelles, solid particles and capsules to contain and protect a drug and deliver it to the site of action either by injection directly into the diseased tissue or by circulation through the blood stream. At the site of action, the enzyme will degrade the polymer, cause disassembly of the micelle or swell the particle and thus cause release of the drug into the environment.

Small bioactive molecules and proteins were loaded into polymeric micelles and released after the enzyme-induced disassembly of the a self-assembled structure (Ge *et al*., 2011; Wang *et al*., 2010). Similarly, proteins were entrapped in the hollow centre of polymer capsules from where they could be released after enzymatic degradation of the capsule (Itoh *et al*., 2006). Proteins have also been encapsulated within a polymer directly. The polymer forms a close shell around each individual protein; that shell can be enzymatically degraded to restore the activity of the protein (Gu *et al*.,

2009). Polymer hydrogel particles have been designed such that exposure to the triggering enzymes causes swelling of the particle. The resulting increase in the mesh size of the polymer hydrogel allows the encapsulated proteins to diffuse out of the particle (McDonald *et al*., 2009; Patrick and Ulijn, 2010; Thornton *et al*., 2005, 2008). An example of this is displayed in Fig. 6.14c. Here, hydrogel particles were prepared from PEGA via an inverse suspension polymerisation. Figure 6.14 C1 shows an environmental scanning electron microscope (ESEM) image of the resulting hydrogel particles. A short peptide sequence with ionisable side groups (Fmoc-Asp-Ala-Ala-Arg) was attached to amine groups incorporated into the polymer. Through pH induced swelling – the appended peptide sequence becomes positively charged at acidic pH causing electrostatic repulsion of neighbouring peptides and resulting in an increase in the mesh size of the hydrogel – the particles were loaded with a fluorescently labelled payload (FITC-dextran) which was trapped inside the particles after the return to a neutral pH and were visualised by florescence microscopy (Fig. 6.14 C2). The peptide sequences were designed such that enzymatic cleavage by thermolysin (cleavage between Aa-Ala) changes the overall charge neutral peptide into an overall positively charged, truncated sequence (Ala-Arg). This causes the particles to swell and allows the payload to escape, resulting in a loss of fluorescence intensity of the particles (Fig. 6.14 C3). (McDonald *et al*., 2009) Besides polymer particles, self-immolative polymers have been used as drug carriers as well. The bioactive agents were attached to the polymer via metastable linkers that become undone at the same time as the polymer disassembles after its exposure to the enzymatic trigger (Shamis *et al*., 2004).

It has already been mentioned above that 'bulk' PEG-based hydrogels, designed as cell culture supports, have simultaneously been used as delivery systems for growth factors (Ehrbar *et al*., 2007). Other PEG hydrogels were used to release proteins upon enzymatic degradation of the material (Aimetti *et al*., 2009; Kurisawa *et al*., 2010).

Dual-responsive polymer-based drug delivery vehicles offer even more versatility. Both temperature and enzyme-responsive micelles were formed from block polymers. While the thermoresponsive properties of the material allow it to be injected and subsequently form a reservoir in the body from where micelles enter the blood stream, the enzyme-responsive component allows triggered degradation of the micelles and release of the drug at the location of the diseased tissue (Garripelli *et al*., 2011). A doubly enzyme-responsive system can be obtained, for example, by designing polymer capsules with a shell that contains two layers. The two layers are degraded by different enzymes; thus, proteins entrapped in the outer layer itself can be released upon exposure to the first enzyme, whereas the second enzyme destroys the capsule completely and liberates the proteins entrapped in the cavity of the vehicle (Itoh *et al*., 2008).

6.7 Conclusion

By definition, ERMs are very well suited to bridge the gap between man-made materials and biological processes (see Table 6.1) (Ulijn, 2006; Zelzer *et al*., 2013). Responsiveness to enzymes provides not only a means of communication between the material and its biological environment, it also presents the possibility of designing materials that display reversible and dynamic responses to a stimulus. While reversibility is not an uncommon feature for smart polymers, a dynamic interaction wherein response of a material only persists in the presence of the stimulus or a cofactor is rare. Several reversible enzyme-responsive polymer systems have been prepared so far (Ku *et al*., 2011), but truly dynamic or fuelled polymer-based systems have yet to be developed.

The predisposition of ERMs to be used as biomaterials comes with some limitations. As the material relies on enzymes as stimuli, the operating conditions must preserve the function of the enzyme. This typically restricts the use of enzyme-responsive polymers to applications that respond under these circumstances. Although several enzymes are known that operate outside these conditions (e.g., at elevated temperatures or in organic solvents), no stimuli-responsive materials have yet been developed that respond to these enzymes. Moreover, since the application of ERMs is focused on biological systems, the polymers used have to be biocompatible and be able to withstand the effects of a complex biological environment in which they have to maintain their function.

With the exception of polymers derived from natural sources, many of the polymers used in ERMs are not enzyme responsive themselves. Enzyme-sensitive functionalities have to be built into the polymers and linked with

Table 6.1 Advantages and limitations of enzyme-responsive polymers

Advantages	Limitations
Enzymes occur naturally	Require physiological conditions
Enzymatic reactions are specific	
The chemistry to incorporate enzyme-sensitive functional groups is simple and readily available	Apart from naturally derived materials, polymers are not enzyme sensitive themselves – enzyme-sensitive functionalities have to be introduced separately
Typical enzyme-sensitive groups (DNA, peptides) are very versatile and can be easily tuned to match an enzyme using the same chemical procedures	
A large number of enzyme/substrate pairs are available	Polymers have to be biocompatible and resistant to the complex biological environment
Responsiveness of one material to more than one enzyme is possible	
Transitions are reversible	

the material such that the enzymatic reaction causes a material response. While this may include additional steps in the preparation of the enzyme-responsive polymer compared to other stimuli-responsive polymers, a range of simple and established reactions are available to accomplish this task. Moreover, the separate addition of enzyme-sensitive functionalities bestows a unique versatility upon the material; the peptide sequences and DNA strands used as enzyme-responsive units can be readily modified to tune the responsiveness of the material to a variety of enzymes. A large choice of enzyme/substrate pairs is known and has been used to design ERMs, providing possibilities for tailored responsiveness that is unprecedented in any other stimuli-responsive material. This unique feature also enables the presence of more than one type of enzyme-responsive functionality to be built into the material, and therefore also sets ERMs apart from other systems such as temperature- or light-responsive materials that require a combination of two different types of stimuli to display two separate response patterns.

6.8 Future trends

In the early stages of the development of ERMs, the systems were mostly designed to be either formed or degraded as a result of the enzymatic reaction (Kurisawa *et al*., 1997; Sperinde and Griffith, 1997). In recent years, it has been recognised that the full potential of enzymes as stimuli for smart materials has not even begun to be explored in depth. By observing and understanding natural processes that are truly reversible and dynamic, it becomes clear that enzyme responsiveness can be much more powerful than the simple degradation of a material. Work on enzyme-responsive polymers is thus beginning to focus more on the reversibility of the response, exploiting, for example, the antagonistic action of two enzymes that catalyse opposite reactions to reversibly change the functionality of a polymer structure (Hahn and Gianneschi, 2011; Zelzer *et al*., 2013). Following this line of thought, it would be very attractive to create fuelled systems wherein the enzyme is able to maintain the material response in the presence of a cofactor that is consumed during the enzymatic reaction. If the fuel is depleted, the enzyme is unable to perform its function and the material reverts back to its original state, until more fuel is provided. Such enzyme-responsive systems have not yet been developed but are likely to be at the centre of intense research in the near future.

It has already been shown that existing enzyme-responsive polymer systems can be integrated with responsiveness to other stimuli (Garripelli *et al*., 2011). It is anticipated that this trend will also continue and enzyme-responsive polymers will find themselves operating along other stimuli-responsive

systems to provide more versatile and complex materials that will suit ever more demanding applications.

Finally, the claim that enzyme-responsive polymers are highly suited for biomedical applications has been frequently justified by model studies including both *in vitro* and *in vivo* work (Jin *et al.*, 2010; Kurisawa *et al.*, 2005). In order to truly exploit the potential of these materials, work that facilitates the translation of these materials into applications needs to be intensified. It is expected that the demand for a stronger drive towards the development of enzyme-responsive polymers into commercially viable materials will be recognised in the near future, and that we will see an increase in the effort to drive the research in this area forward.

6.9 References

Aimetti, A. A., Machen, A. J. and Anseth, K. S. (2009). Poly(ethylene glycol) hydrogels formed by thiol-ene photopolymerization for enzyme-responsive protein delivery. *Biomaterials*, **30**, 6048–6054.

Alarcon, C. D. H., Pennadam, S. and Alexander, C. (2005). Stimuli responsive polymers for biomedical applications. *Chemical Society Reviews,* **34**, 276–285.

Alemdaroglu, F. E., Wang, J., Borsch, M., Berger, R. and Herrmann, A. (2008). Enzymatic control of the size of DNA block copolymer nanoparticles. *Angewandte Chemie-International Edition,* **47**, 974–976.

Amir, R. J., Pessah, N., Shamis, M. and Shabat, D. (2003). Self-immolative dendrimers. *Angewandte Chemie-International Edition,* **42**, 4494–4499.

Amir, R. J., Zhong, S., Pochan, D. J. and Hawker, C. J. (2009). Enzymatically triggered self-assembly of block copolymers. *Journal of the American Chemical Society,* **131**, 13949–13951.

Andrieu, J., Kotman, N., Maier, M., Mailander, V., Strauss, W. S. L., Weiss, C. K. and Landfester, K. (2012). Live monitoring of cargo release from peptide-based hybrid nanocapsules induced by enzyme cleavage. *Macromolecular Rapid Communications,* **33**, 248–253.

Azagarsamy, M. A., Sokkalingam, P. and Thayumanavan, S. (2009). Enzyme-triggered disassembly of dendrimer-based amphiphilic nanocontainers. *Journal of the American Chemical Society,* **131**, 14184–14185.

Biswas, A., Joo, K. I., Liu, J., Zhao, M. X., Fan, G. P., Wang, P., Gu, Z. and Tang, Y. (2011). Endoprotease-mediated intracellular protein delivery using nanocapsules. *Acs Nano,* **5**, 1385–1394.

Blencowe, C. A., Russell, A. T., Greco, F., Hayes, W. and Thornthwaite, D. W. (2011). Self-immolative linkers in polymeric delivery systems. *Polymer Chemistry,* **2**, 773–790.

Börner, H. G., Kühnle, H. and Hentschel, J. (2010). Making 'smart polymers' smarter: Modern concepts to regulate functions in polymer science. *Journal of Polymer Science Part a-Polymer Chemistry,* **48**, 1–14.

Caponi, P.-F., Qiu, X.-P., Vilela, F., Winnik, F. M. and Ulijn, R. V. (2011). Phosphatase/temperature responsive poly(2-isopropyl-2-oxazoline). *Polymer Chemistry,* **2**, 306–308.

Castelletto, V., Mckendrick, J. E., Hamley, I. W., Olsson, U. and Cenker, C. 2010. PEGylated amyloid peptide nanocontainer delivery and release system. *Langmuir,* **26**, 11624–11627.

Chen, T. H., Embree, H. D., Wu, L. Q. and Payne, G. F. (2002). In vitro protein-polysaccharide conjugation: Tyrosinase-catalyzed conjugation of gelatin and chitosan. *Biopolymers,* **64**, 292–302.

Chien, M. P., Rush, A. M., Thompson, M. P. and Gianneschi, N. C. (2010). Programmable shape-shifting micelles. *Angewandte Chemie-International Edition,* **49**, 5076–5080.

Cole, M. A., Voelcker, N. H., Thissen, H. and Griesser, H. J. (2009). Stimuli-responsive interfaces and systems for the control of protein-surface and cell-surface interactions. *Biomaterials,* **30**, 1827–1850.

Crespy, D. and Landfester, K. (2010). Miniemulsion polymerization as a versatile tool for the synthesis of functionalized polymers. *Beilstein Journal of Organic Chemistry,* **6**, 1132–1148.

de Graaf, A. J., Mastrobattista, E., Vermonden, T., VAN Nostrum, C. F., Rijkers, D. T. S., Liskamp, R. M. J. and Hennink, W. E. (2012). Thermosensitive peptide-hybrid ABC block copolymers obtained by ATRP: Synthesis, self-assembly, and enzymatic degradation. *Macromolecules,* **45**, 842–851.

de Groot, F. M. H., Albrecht, C., Koekkoek, R., Beusker, P. H. and Scheeren, H. W. (2003). 'Cascade-release dendrimers' liberate all end groups upon a single triggering event in the dendritic core. *Angewandte Chemie-International Edition,* **42**, 4490–4494.

Delcea, M., Mohwald, H. and Skirtach, A. G. (2011). Stimuli-responsive LbL capsules and nanoshells for drug delivery. *Advanced Drug Delivery Reviews,* **63**, 730–747.

Ehrbar, M., Rizzi, S. C., Schoenmakers, R. G., San Miguel, B., Hubbell, J. A., Weber, F. E. and Lutolf, M. P. (2007). Biomolecular hydrogels formed and degraded via site-specific enzymatic reactions. *Biomacromolecules,* **8**, 3000–3007.

Fedorovich, N. E., Alblas, J., De Wijn, J. R., Hennink, W. E., Verbout, A. J. and Dhert, W. J. A. (2007). Hydrogels as extracellular matrices for skeletal tissue engineering: state-of-the-art and novel application in organ printing. *Tissue Engineering,* **13**, 1905–1925.

Folk, J. E. and Finlayson, J. S. (1977). The epsilon-(gamma-glutamyl) lysine crosslink and the catalytic role of transglutaminases. *Advances in protein chemistry,* **31**, 1–133.

Fuchsbauer, H. L., Gerber, U., Engelmann, J., Seeger, T., Sinks, C. and Hecht, T. (1996). Influence of gelatin matrices cross-linked with transglutaminase on the properties of an enclosed bioactive material using beta-galactosidase as model system. *Biomaterials,* **17**, 1481–1488.

Garripelli, V. K., Kim, J. K., Son, S., Kim, W. J., Repka, M. A. and Jo, S. (2011). Matrix metalloproteinase-sensitive thermogelling polymer for bioresponsive local drug delivery. *Acta Biomaterialia,* **7**, 1984–1992.

Ge, J., Lu, D. N., Yang, C. and Liu, Z. (2011). A lipase-responsive vehicle using amphipathic polymer synthesized with the lipase as catalyst. *Macromolecular Rapid Communications,* **32**, 546–550.

Ghadiali, J. E. and Stevens, M. M. (2008). Enzyme-responsive nanoparticle systems. *Advanced Materials,* **20**, 4359–4363.

Glangchai, L. C., Caldorera-Moore, M., Shi, L. and Roy, K. (2008). Nanoimprint lithography based fabrication of shape-specific, enzymatically-triggered smart nanoparticles. *Journal of Controlled Release,* **125**, 263–272.

Gu, Z., Yan, M., Hu, B. L., Joo, K. I., Biswas, A., Huang, Y., Lu, Y. F., Wang, P. and Tang, Y. (2009). Protein nanocapsule weaved with enzymatically degradable polymeric network. *Nano Letters,* **9**, 4533–4538.

Guan, J., Fujimoto, K. L. and Wagner, W. R. (2008). Elastase-sensitive elastomeric scaffolds with variable anisotropy for soft tissue engineering. *Pharmaceutical Research,* **25**, 2400–2412.

Haba, K., Popkov, M., Shamis, M., Lerner, R. A., Barbas, C. F. and Shabat, D. (2005). Single-triggered trimeric prodrugs. *Angewandte Chemie-International Edition,* **44**, 716–720.

Habraken, G. J. M., Peeters, M., Thornton, P. D., Koning, C. E. and Heise, A. (2011). Selective enzymatic degradation of self-assembled particles from amphiphilic block copolymers obtained by the combination of n-carboxyanhydride and nitroxide-mediated polymerization. *Biomacromolecules,* **12**, 3761–3769.

Hahn, M. E. and Gianneschi, N. C. (2011). Enzyme-directed assembly and manipulation of organic nanomaterials. *Chemical Communications,* **47**, 11814–11821.

Itoh, Y., Matsusaki, M., Kida, T. and Akashi, M. (2006). Enzyme-responsive release of encapsulated proteins from biodegradable hollow capsules. *Biomacromolecules,* **7**, 2715–2718.

Itoh, Y., Matsusaki, M., Kida, T. and Akashi, M. (2008). Time-modulated release of multiple proteins from enzyme-responsive multilayered capsules. *Chemistry Letters,* **37**, 238–239.

Jin, R., Hiemstra, C., Zhong, Z. Y. and Feijen, J. (2007). Enzyme-mediated fast in situ formation of hydrogels from dextran-tyramine conjugates. *Biomaterials,* **28**, 2791–2800.

Jin, R., Teixeira, L. S. M., Dijkstra, P. J., van Blitterswijk, C. A., Karperien, M. and Feijen, J. (2010). Enzymatically-crosslinked injectable hydrogels based on biomimetic dextran-hyaluronic acid conjugates for cartilage tissue engineering. *Biomaterials,* **31**, 3103–3113.

Kanaras, A. G., Wang, Z. X., Bates, A. D., Cosstick, R. and Brust, M. (2003). Towards multistep nanostructure synthesis: Programmed enzymatic self-assembly of DNA/gold systems. *Angewandte Chemie-International Edition,* **42**, 191–194.

Khelfallah, N. S., Decher, G. and Mesini, P. J. (2006). Synthesis of a new PHEMA/PEO enzymatically biodegradable hydrogel. *Macromolecular Rapid Communications,* **27**, 1004–1008.

Kim, S. and Healy, K. E. (2003). Synthesis and characterization of injectable poly(N-isopropylacrylamide-co-acrylic acid) hydrogels with proteolytically degradable cross-links. *Biomacromolecules,* **4**, 1214–1223.

Klinger, D., Aschenbrenner, E. M., Weiss, C. K. and Landfester, K. (2012). Enzymatically degradable nanogels by inverse miniemulsion copolymerization of acrylamide with dextran methacrylates as crosslinkers. *Polymer Chemistry,* **3**, 204–216.

Koga, H., Toita, R., Mori, T., Tomiyama, T., Kang, J. H., Niidome, T. and Katayama, Y. (2011). Fluorescent nanoparticles consisting of lipopeptides and fluorescein-modified polyanions for monitoring of protein kinase activity. *Bioconjugate Chemistry,* **22**, 1526–1534.

Ku, T. H., Chien, M. P., Thompson, M. P., Sinkovits, R. S., Olson, N. H., Baker, T. S. and Gianneschi, N. C. (2011). Controlling and switching the morphology of micellar nanoparticles with enzymes. *Journal of the American Chemical Society,* **133**, 8392–8395.

Kühnle, H. and Börner, H. G. (2009). Biotransformation on polymer-peptide conjugates: A versatile tool to trigger microstructure formation. *Angewandte Chemie-International Edition,* **48**, 6431–6434.

Kumashiro, Y., Lee, T. K., Ooya, T. and Yui, N. (2002). Enzymatic degradation of semi-IPN hydrogels based on N-isopropylacrylamide and dextran at a specific temperature range. *Macromolecular Rapid Communications,* **23**, 407–410.

Kurisawa, M., Chung, J. E., Yang, Y. Y., Gao, S. J. and Uyama, H. (2005). Injectable biodegradable hydrogels composed of hyaluronic acid-tyramine conjugates for drug delivery and tissue engineering. *Chemical Communications*, **34**, 4312–4314.

Kurisawa, M., Lee, F., Wang, L. S. and Chung, J. E. (2010). Injectable enzymatically crosslinked hydrogel system with independent tuning of mechanical strength and gelation rate for drug delivery and tissue engineering. *Journal of Materials Chemistry,* **20**, 5371–5375.

Kurisawa, M., Terano, M. and Yui, N. (1997). Double-stimuli-responsive degradation of hydrogels consisting of oligopeptide-terminated poly(ethylene glycol) and dextran with an interpenetrating polymer network. *Journal of Biomaterials Science-Polymer Edition,* **8**, 691–708.

Lehn, J. M. (2002). Toward complex matter: Supramolecular chemistry and self-organization. *Proceedings of the National Academy of Sciences of the United States of America,* **99**, 4763–4768.

Lutolf, M. P. and Hubbell, J. A. (2003). Synthesis and physicochemical characterization of end-linked poly(ethylene glycol)-co-peptide hydrogels formed by Michael-type addition. *Biomacromolecules,* **4**, 713–722.

Lutolf, M. P. and Hubbell, J. A. (2005). Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nature Biotechnology,* **23**, 47–55.

Lutolf, M. P., Lauer-Fields, J. L., Schmoekel, H. G., Metters, A. T., Weber, F. E., Fields, G. B. and Hubbell, J. A. (2003a). Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: Engineering cell-invasion characteristics. *Proceedings of the National Academy of Sciences of the United States of America,* **100**, 5413–5418.

Lutolf, M. P., Raeber, G. P., Zisch, A. H., Tirelli, N. and Hubbell, J. A. (2003b). Cell-responsive synthetic hydrogels. *Advanced Materials,* **15**, 888–892.

Maier, M., Kotman, N., Friedrichs, C., Andrieu, J., Wagner, M., Graf, R., Strauss, W. S. L., Mailander, V., Weiss, C. K. and Landfester, K. (2011). Highly site specific, protease cleavable, hydrophobic peptide-polymer nanoparticles. *Macromolecules,* **44**, 6258–6267.

Mann, B. K., Gobin, A. S., Tsai, A. T., Schmedlen, R. H. and West, J. L. (2001). Smooth muscle cell growth in photopolymerized hydrogels with cell adhesive and proteolytically degradable domains: synthetic ECM analogs for tissue engineering. *Biomaterials,* **22**, 3045–3051.

Mano, J. F. (2008). Stimuli-responsive polymeric systems for biomedical applications. *Advanced Engineering Materials,* **10**, 515–527.

Mcdonald, T. O., Qu, H. L., Saunders, B. R. and Ulijn, R. V. (2009). Branched peptide actuators for enzyme responsive hydrogel particles. *Soft Matter,* **5**, 1728–1734.

Metcalfe, A. D. and Ferguson, M. W. J. (2008). Skin stem and progenitor cells: using regeneration as a tissue-engineering strategy. *Cellular and Molecular Life Sciences,* **65**, 24–32.

Miyata, T., Uragami, T. and Nakamae, K. (2002). Biomolecule-sensitive hydrogels. *Advanced Drug Delivery Reviews,* **54**, 79–98.

Morimoto, N., Ogino, N., Narita, T., Kitamura, S. and Akiyoshi, K. (2007). Enzyme-responsive molecular assembly system with amylose-primer surfactants. *Journal of the American Chemical Society,* **129**, 458–459.

Mutter, M., Chandravarkar, A., Boyat, C., Lopez, J., Dos Santos, S., Mandal, B., Mimna, R., Murat, K., Patiny, L., Saucede, L. and Tuchscherer, G. (2004). Switch peptides in statu nascendi: Induction of conformational transitions relevant to degenerative diseases. *Angewandte Chemie-International Edition,* **43**, 4172–4178.

Patrick, A. G. and Ulijn, R. V. (2010). Hydrogels for the detection and management of protease levels. *Macromolecular Bioscience,* **10**, 1184–1193.

Phelps, E. A., Landazuri, N., Thule, P. M., Taylor, W. R. and Garcia, A. J. (2010). Bioartificial matrices for therapeutic vascularization. *Proceedings of the National Academy of Sciences of the United States of America,* **107**, 3323–3328.

Raghupathi, K. R., Azagarsamy, M. A. and Thayumanavan, S. (2011). Guest-release control in enzyme-sensitive, amphiphilic-dendrimer-based nanoparticles through photochemical crosslinking. *Chemistry-a European Journal,* **17**, 11752–11760.

Rawsterne, R. E., Gough, J. E., Rutten, F. J. M., Pham, N. T., Poon, W. C. K., Flitsch, S. L., Maltman, B., Alexander, M. R. and Ulijn, R. V. (2006). Controlling protein retention on enzyme-responsive surfaces. *Surface and Interface Analysis,* **38**, 1505–1511.

Riess, G. (2003). Micellization of block copolymers. *Progress in Polymer Science,* **28**, 1107–1170.

Roy, S. and Ulijn, R. V. (2010). Exploiting biocatalysis in the synthesis of supramolecular polymers. *In:* Palmans, A. R. A. and Heise, A. (eds.) *Enzymatic Polymerisation.* Berlin: Springer-Verlag Berlin.

Sakai, S., Hirose, K., Taguchi, K., Ogushi, Y. and Kawakami, K. (2009a). An injectable, in situ enzymatically gellable, gelatin derivative for drug delivery and tissue engineering. *Biomaterials,* **30**, 3371–3377.

Sakai, S. and Kawakami, K. (2007). Synthesis and characterization of both ionically and enzymatically cross-linkable alginate. *Acta Biomaterialia,* **3**, 495–501.

Sakai, S., Ogushi, Y. and Kawakami, K. (2009b). Enzymatically crosslinked carboxymethylcellulose-tyramine conjugate hydrogel: Cellular adhesiveness and feasibility for cell sheet technology. *Acta Biomaterialia,* **5**, 554–559.

Sanborn, T. J., Messersmith, P. B. and Barron, A. E. (2002). In situ crosslinking of a biomimetic peptide-PEG hydrogel via thermally triggered activation of factor XIII. *Biomaterials,* **23**, 2703–2710.

Schork, F. J., Luo, Y. W., Smulders, W., Russum, J. P., Butte, A. and Fontenot, K. (2005). Miniemulsion polymerization. *In:* Okubo, M. (ed.) *Polymer Particles.* Berlin: Springer-Verlag Berlin.

Shamis, M., Lode, H. N. and Shabat, D. (2004). Bioactivation of self-immolative dendritic prodrugs by catalytic antibody 38C2. *Journal of the American Chemical Society,* **126**, 1726–1731.

Shoichet, M. S. (2010). Polymer scaffolds for biomaterials applications. *Macromolecules,* **43**, 581–591.

Sperinde, J. J. and Griffith, L. G. (1997). Synthesis and characterization of enzymatically-cross-linked poly(ethylene glycol) hydrogels. *Macromolecules,* **30**, 5255–5264.

Stuart, M. A. C., Huck, W. T. S., Genzer, J., Muller, M., Ober, C., Stamm, M., Sukhorukov, G. B., Szleifer, I., Tsukruk, V. V., Urban, M., Winnik, F., Zauscher, S., Luzinov, I. and Minko, S. (2010). Emerging applications of stimuli-responsive polymer materials. *Nature Materials,* **9**, 101–113.

Sun, T. L., Qing, G. Y., Su, B. L. and Jiang, L. (2011). Functional biointerface materials inspired from nature. *Chemical Society Reviews,* **40**, 2909–2921.

Szalai, M. L., Kevwitch, R. M. and Mcgrath, D. V. (2003). Geometric disassembly of dendrimers: Dendritic amplification. *Journal of the American Chemical Society,* **125**, 15688–15689.

Thornton, P. D., Mart, R. J., Webb, S. J. and Ulijn, R. V. (2008). Enzyme-responsive hydrogel particles for the controlled release of proteins: designing peptide actuators to match payload. *Soft Matter,* **4**, 821–827.

Thornton, P. D., Mcconnell, G. and Ulijn, R. V. (2005). Enzyme responsive polymer hydrogel beads. *Chemical Communications*, (**47**) 5913–5915 .

Tibbitt, M. W. and Anseth, K. S. (2009). Hydrogels as extracellular matrix mimics for 3D cell culture. *Biotechnology and Bioengineering,* **103**, 655–663.

Ulijn, R. V. (2006). Enzyme-responsive materials: a new class of smart biomaterials. *Journal of Materials Chemistry,* **16**, 2217–2225.

Van Dijk, M., Van Nostrum, C. F., Hennink, W. E., Rijkers, D. T. S. and Liskamp, R. M. J. (2010). Synthesis and characterization of enzymatically biodegradable PEG and peptide-based hydrogels prepared by click chemistry. *Biomacromolecules,* **11**, 1608–1614.

Wang, C., Chen, Q. S., Wang, Z. Q. and Zhang, X. (2010). An enzyme-responsive polymeric superamphiphile. *Angewandte Chemie-International Edition,* **49**, 8612–8615.

Weinstain, R., Baran, P. S. and Shabat, D. (2009). Activity-linked labeling of enzymes by self-immolative polymers. *Bioconjugate Chemistry,* **20**, 1783–1791.

Weinstain, R., Sagi, A., Karton, N. and Shabat, D. (2008). Self-immolative comb-polymers: Multiple-release of side-reporters by a single stimulus event. *Chemistry-a European Journal,* **14**, 6857–6861.

Wen, J., Anderson, S. M., Du, J. J., Yan, M., Wang, J., Shen, M. Q., Lu, Y. F. and Segura, T. (2011). Controlled protein delivery based on enzyme-responsive nanocapsules. *Advanced Materials,* **23**, 4549–4553.

West, J. L. and Hubbell, J. A. (1999). Polymeric biomaterials with degradation sites for proteases involved in cell migration. *Macromolecules,* **32**, 241–244.

Whyte, M. P. (2010). Physiological role of alkaline phosphatase explored in hypophosphatasia. *In:* Zaidi, M. (ed.) *Skeletal Biology and Medicine.* Oxford: Blackwell Publishing.

Wichterle, O. and Lim, D. (1960). Hydrophilic gels for biological use. *Nature,* **185**, 117–118.

Woodcock, J. W., Jiang, X. G., Wright, R. A. E. and Zhao, B. (2011). Enzyme-induced formation of thermoreversible micellar gels from aqueous solutions of multiresponsive hydrophilic ABA triblock copolymers. *Macromolecules,* **44**, 5764–5775.

Yamamoto, N., Kurisawa, M. and Yui, N. (1996). Double-stimuli-responsive degradable hydrogels: Interpenetrating polymer networks consisting of gelatin and dextran with different phase separation. *Macromolecular Rapid Communications,* **17**, 313–318.

Yang, J. Y., Jacobsen, M. T., Pan, H. Z. and Kopecek, J. (2010). Synthesis and characterization of enzymatically degradable PEG-based peptide-containing hydrogels. *Macromolecular Bioscience,* **10**, 445–454.

Yang, Z. M., Gu, H. W., Fu, D. G., Gao, P., Lam, J. K. and Xu, B. (2004). Enzymatic formation of supramolecular hydrogels. *Advanced Materials,* **16**, 1440–1444.

Yang, Z. M., Liang, G. L., Wang, L. and Xu, B. (2006). Using a kinase/phosphatase switch to regulate a supramolecular hydrogel and forming the supramolecular hydrogel in vivo. *Journal of the American Chemical Society,* **128**, 3038–3043.

Yeo, W. S. and Mrksich, M. (2003). Self-assembled monolayers that transduce enzymatic activities to electrical signals. *Angewandte Chemie-International Edition,* **42**, 3121–3124.

Zelzer, M., Todd, S. J., Hirst, A. R., Mcdonald, T. O. and Ulijn, R. V. (2013). Enzyme responsive materials: Design strategies and future developments. *Biomaterials Science,* **1**, 11–39.

Zhao, M., Josephson, L., Tang, Y. and Weissleder, R. (2003). Magnetic sensors for protease assays. *Angewandte Chemie-International Edition,* **42**, 1375–1378.

Zisch, A. H., Lutolf, M. P., Ehrbar, M., Raeber, G. P., Rizzi, S. C., Davies, N., Schmokel, H., Bezuidenhout, D., Djonov, V., Zilla, P. and Hubbell, J. A. (2003). Cell-demanded release of VEGF from synthetic, biointeractive cell-ingrowth matrices for vascularized tissue growth. *Faseb Journal,* **17**, 2260–2262.

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Shape memory polymers: properties, synthesis and applications

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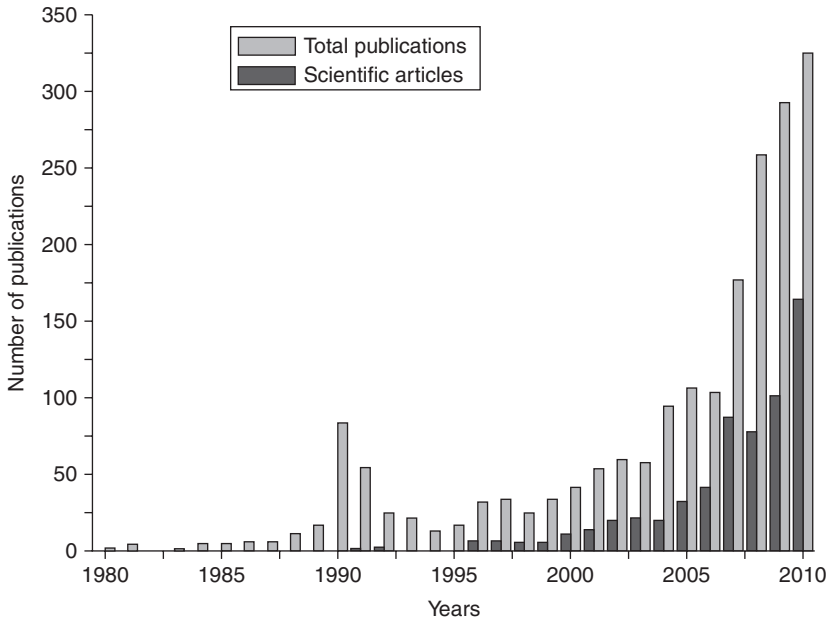
Abstract: Shape memory materials are able to change their shape in a controlled way upon application of an external stimulus such as temperature, light, application of electric or magnetic fields, etc. Due to their scientific and technological relevance, our aim is to review shape memory polymers, briefly introducing metals and ceramics. Polymeric materials show a wide range of relevant properties such as processability, versatility and biocompatibility, among others, characteristics that are responsible for the amplified interest in recent years in the shape memory field. With their cheaper cost and wide application spectrum, this review also reports the numerous advantages of shape memory polymers over alloys and ceramics, which are driving current research trends.

Key words: shape memory materials, shape memory polymers, external stimuli activation, biomedical applications.

7.1 Introduction

Shape memory materials (SMMs) comprise a class of smart materials able to change their shape following application of an external stimulus. The first materials studied with shape memory behaviour were developed in the middle of the twentieth century. Since then, research into and demand for these materials have both increased, especially in recent years, due to their unique properties, versatility and growing industrial demands. Figure 7.1 shows the total number of scientific publications indexed on ISI Web of Knowledge and the number of research articles about shape memory polymers (SMPs) in the last 30 years; in particular, the increase during the last six years is significant.

SMMs are able to respond to an external stimulus changing their own structure. The common external stimuli used to provoke the shape memory effects (SMEs) can be temperature (Knight *et al*, 2009; Lendlein *et al*.,

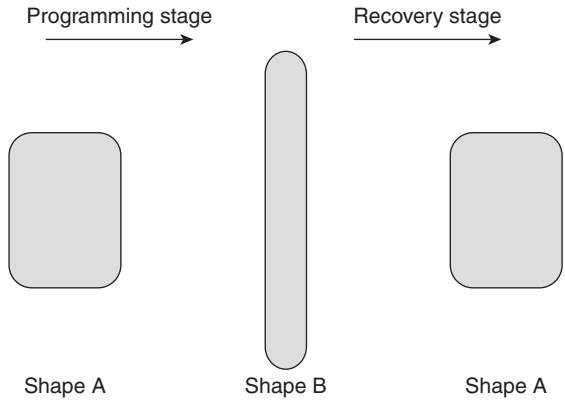


7.1 ISI publications referring to polymers with shape memory behaviour. (*Source*: ISI Web of Knowledge.)

2001; Liu and Cai, 2009a; Nagata *et al*., 2010), light (Ahir and Terentejev, 2005; Vaia, 2005; Yu and Ikeda, 2005), pH (Lee *et al*., 1996; Markland *et al*., 1999; Osada and Gong, 1998), the application of magnetic (Mohr *et al*., 2006; Schmidt, 2006; Zheng *et al*, 2009) and electrical fields (Cho *et al*., 2005; Leng *et al*., 2007; Liu *et al*., 2009b) and contact with some solvents (Huang *et al*., 2010; Yang *et al*., 2004). The potential use of SMMs covers different fields such as the aerospace industry (Arzberger *et al*., 2005; Gall *et al*., 2000), mechatronic engineering (Monkman, 2000) and the biomedical sector (Karp and Langer, 2007; Lendlein and Langer, 2002; Nji and Li, 2010; Song *et al*., 2010b).

Many kinds of materials can show shape memory behaviour such as metal alloys (Chau *et al*., 2006; Gil *et al*., 2004; He *et al*., 2005; Rossi *et al*., 2008; Van Humbeeck, 1999), ceramics (Heuer *et al*., 1990; Wang *et al*., 1991) and polymers (Lendlein *et al*., 2004; Wei *et al*., 1998) and, due to the different structure of these materials, the shape memory mechanism is different in each case.

Usually 'shape memory' refers to materials with 'one-way shape memory effect' behaviour (Fig. 7.2). This process comprises two stages known as programming and recovery. In 'programming', a 'temporary shape' is fixed from the 'fixity shape'. This mechanism is different in metal alloys, ceramics



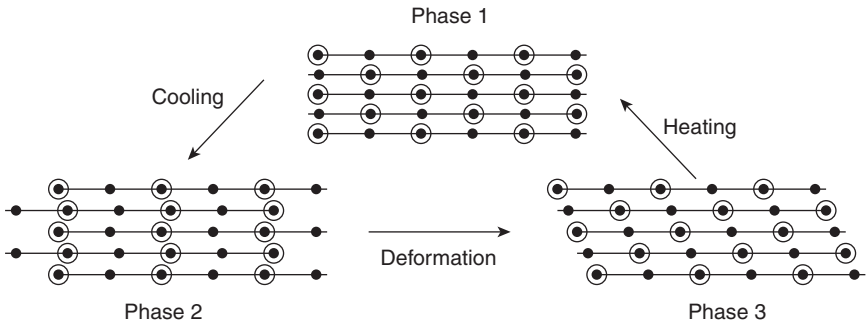
7.2 Stages of 'one-way shape memory effect'.

or polymers. In the second stage, the 'fixed shape' is recovered from the 'temporary shape'. For instance, if the external stimulus is temperature, the recovery consists of heating the material above its transition temperature (T_{trans}). Other types of shape memory response reported are 'two-way shape memory effect' (Chen *et al.*, 2008; Chen *et al.*, 2010; Wada and Liu, 2008) and 'multi-shape memory effect' (Luo *et al.*, 2008; Xie, 2010). The former is a reversible transition between two different shapes of the material, one of these occurring when the temperature is below T_{trans}, while the other shape is obtained by increasing the temperature above T_{trans}. Materials with 'multi-shape memory effect' are capable of 'remembering' more than one temporary shape, taking advantage of the occurrence of more than one transition temperature. During the programming phase, the temporary shapes can be designed according to each transition temperature, while in the recovery process the materials progressively recover their temporary shapes, until the fixity shape is reached.

In this review, metal alloys and ceramics with shape memory effect will be briefly analysed focusing attention on the different mechanisms responsible for their shape fixity and on their main applications. Then, SMPs will be present, paying attention to their classification, based on the micro-structure of the polymer and on the stimulus capable of activating the recovery phases.

7.1.1 Metal alloys

Shape memory alloys were the first SMMs studied. Chang and Read (1951) developed the first shape memory alloy, composed from gold and cadmium. However, only after 1963, when an equiatomic nickel–titanium alloy called Nitinol was developed (Buehler *et al.*, 1963), were shape memory



7.3 Shape memory effect in metallic alloys. Martensitic transition diffusion.

alloys extensively studied. The shape memory effect in the metallic alloys is achieved through *diffusionless martensitic transition* (Hornbogen, 2006). This transformation leads to a phase change in the alloy capable of changing the symmetry of the crystalline network. The temperature at which this transformation takes place is named the transition temperature, T_{trans}. When the temperature is above T_{trans}, the alloy is in its austenitic phase with its cubic crystalline symmetry network. When the alloy is cooled below T_{trans}, it reaches the martensitic phase where the atoms are closer. In this phase it is possible to deform and change the shape of the material and it allows fixation of the temporary phase. If the material is reheated above T_{trans}, the alloy will recover its original shape (Fig. 7.3).

Through different thermal treatments it is possible to obtain the two-way shape memory effect in some metallic alloys (Chang *et al.*, 2001; Wada and Liu, 2008; Wei *et al.*, 1998). These alloys can change between two shapes through martensitic transformation and without external stress application. The reversible deformation in these alloys is around 10% in the best cases (Liu *et al.*, 2007; Wei *et al.*, 1998). Nitinol (Buehler *et al.*, 1963; Wada and Liu, 2008), is the shape memory alloy most commonly used, due to its good mechanical properties, easy processability and biocompatibility. Moreover, in this material a little change in the alloy composition causes a big change in the transition temperature allowing control of this temperature in a range from –20°C to 60°C. These properties have determined the wide use of Nitinol in biomedical sector in vascular stents, dental wires (Gil and Hudson, 2004), etc.

Starting from this alloy, ternary alloys with other metals have been obtained. For instance, with alloys made with titanium, nickel and copper, the hysteresis is reduced from 30°C to 5°C (Gil and Hudson, 2004). On the other hand when nickel and titanium are alloyed with palladium, gold, platinum, zirconium or hafnium (Bigelow *et al.*, 2010), their transition

temperature increases, obtaining shape memory alloys with high transition temperature. Finally, when niobium is alloyed to NiTi alloys, shape memory alloy with a large hysteresis, about 150°C, can be obtained (He *et al*., 2005). Due to the high cost of nickel and titanium and the requirements of a large range of transition temperatures in order to cover different applications, shape memory metallic alloys based on copper, iron, zinc and aluminium among others have been developed (Colic *et al*., 2010; Hane and Shield, 1999).

7.1.2 Ceramics

Shape memory ceramics (SMCs) have also been studied. The principal drawback of the SMC materials is their small recovery strain, much smaller than those of metal alloys, due to the intrinsic fragile behaviour and the microfractures that ceramics tend to produce in their structure. However, it is possible to classify these materials in terms of their shape memory mechanisms (Wei *et al*., 1998):

- Martensitic ceramics are the most studied and applied as SMMs (Heuer *et al*., 1990), thanks to the relatively high recoverable strains they present. The mechanism is analogous to the metallic alloys: there is a transition temperature T_{trans} between two crystalline phases of the network allowing the shape changes. Below the transition temperature the ceramic has a monoclinic crystalline network; in this state ceramic is plastically deformable. When the temperature exceeds the transition temperature, the network becomes tetragonal and the ceramic recovers its original shape. For these materials, the maximum strain achieved is not more than 4%. Wang (1991) has studied the shape memory effect in high-temperature superconductor ceramics like yttrium barium copper oxide (YBaCuO). The T_{trans} in this ceramic is slightly above its critical temperature.
- Another type of SMC is represented by viscoelastic ceramics (Schurch and Ashbee, 1977). These ceramics are composed of two phases, one crystalline and the other one amorphous. The crystalline phase is mica, which is dispersed in the vitreous matrix. By heating the material above 300°C the mica can deform thanks to stress application. If the stress remains while the material is cooling below 300°C, the amorphous matrix fixes the temporary phase. When the ceramic is reheated above its transition temperature, it recovers the original shape. Only 1% deformations are possible for these ceramics (Wei *et al*., 1998).
- Finally, in ferroelectric and ferromagnetic ceramics, it is possible to obtain the phase transitions by changing the temperature, leading to the development of paraelectric–ferroelectric or paramagnetic–ferromagnetic

ceramics (Li *et al.*, 2005). This temperature change is responsible for the shape memory mechanism in these ceramics.

7.1.3 Polymer materials with shape memory

The first shape memory effect in polymers was reported in the 1960s (Rainer *et al.*, 1964). This polymer consisted of a matrix of polyethylene irradiated with gamma radiation; the result was a material able to memorize its initial shape. From then on, research in SMPs has increased due to their potential application in several fields such as the biomedical sector, aerospace or textile industries. Shape memory effect has been reported in different types of polymers like thermosets, thermoplastics, elastomers, hydrogels and liquid crystals. The interest aroused for using SMPs is to be found in their easy processability, low cost, softness and their versatility for easy design for specific applications by different techniques such as blending, making copolymers, adding fillers, etc. In their review, Behl and co-workers (2010) introduce SMPs as multifunctional materials due to their multiple properties such as biodegradability, thermal and electrical conductivity, light emission and others.

SMPs allow large, recoverable strains, up to 400% (Knight *et al.*, 2009). This strain can be recovered through the action of an external stimulus, retrieving its initial shape. The possibility of using large strains gives more flexibility for fixing the temporary shape in contrast with metallic alloys and ceramics. Moreover, polymer materials are normally cheaper than the others. As drawbacks, polymers present the worst mechanical properties and they do not tolerate great stresses. So, with the aim of overcoming these drawbacks, researchers have focused their interests in polymer composites based on the addition of fibres, fillers and nanofillers to the polymeric matrix. In their review, Liu and co-workers (2007) report a comparison between SMPs and SMAs. Some of these values are summarized here in Table 7.1 showing the wide contrast between features and costs of metallic alloys, ceramics and polymers. For instance, it is worth noting that strain in polymers is two orders of magnitude bigger than the other two classes of materials.

Shape memory effect in polymers is normally achieved by relaxation of the polymeric chains due to changes in temperature. When the programming and recovery stages in the shape memory effect are produced by changes in temperature, it is said that the effect is thermally induced.

In general, SMPs are formed by two active phases. One is the fixity phase and the other one acts as the 'switch phase'. The fixity phase memorizes the initial shape of the polymer while the switch phase allows fixing the

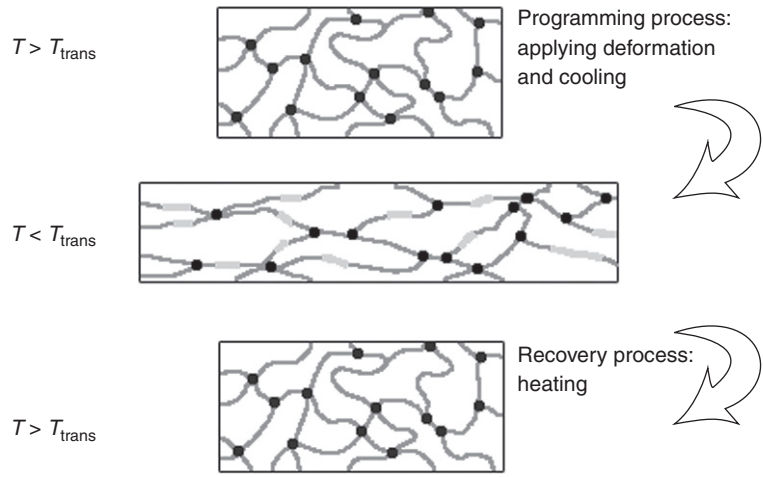
Table 7.1 Comparison of main properties in shape memory materials: metal alloys (SMAs), ceramics (SMCs) and polymers (SMPs)

	SMAs	SMCs	SMPs
Density (g/cm^3)	6–8	2	0.9–1.1
Recoverable strain (%)	< 10%	1–4%	> 400%
Transition temperature (°C)	5–30	300	10–50
Biocompatibility and biodegradability	Can be biocompatible but cannot be biodegradable	Can be biocompatible but cannot be biodegradable	Can be biocompatible and/or biodegradable
Processing conditions	High temperature (>1000°C), high pressures	High temperature	< 200°C, low pressure
Price (€/Kg)	300	>500	15

temporary shape. In addition the switch phase has a transition temperature T_{trans}, which enables the recovery of the primary shape. This temperature can be the melt temperature (T_m) or the glass transition temperature (T_g) of the polymer itself depending on the crystalline or amorphous nature of the switch phase.

The whole shape memory process, schematized in Fig. 7.4, can be summarized in four points:

- The polymer chains are in coil conformation when the polymer is synthesized. The coil is the favourable entropic conformation (Lendlein and Kelch, 2002b).
- The fixity phase prevents the chains slipping through intermolecular or intermolecular bonds (black dots in Fig. 7.4).
- Switching chains enables the shape memory effect. Below the T_{trans} of the switch phase (light grey in Fig. 7.4) the chain movements are not allowed. If the temperature increases above T_{trans}, the chains can move. In this state, if a stress is applied, the chains will suffer an elastic deformation, reducing the entropy of the chain conformation. Releasing the stress, the polymer is able to go back to its original conformation.
- On the other hand, when the temperature is below T_{trans}, the switching phase (light grey in Fig. 7.4) will fix the chains keeping the temporary shape. When temperature is below T_{trans} it is possible to release the stress.
- Finally, when the temperature is increased above T_{trans}, the polymer will recover its original shape, because this shape is entropically more favourable.



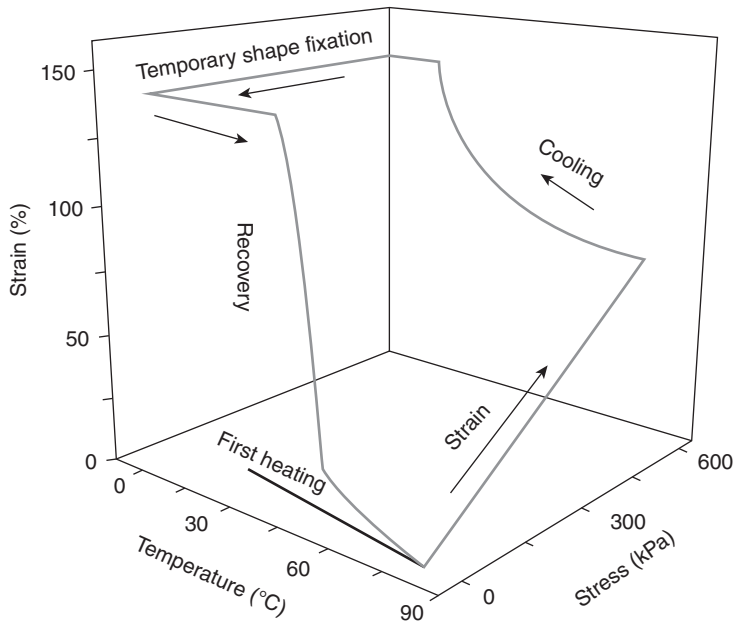
7.4 Shape memory effect in polymers.

7.2 Characterizing shape memory effects in polymeric materials

There are no standard procedures for the characterization of the shape memory effects of polymeric materials; so in order to allow a comparison between different SMMs, the quantification of the shape memory effect is realized through mechanical tests with specific procedures and parameters. In general the procedures described in the literature consist of tensile stress–strain or bending tests with a temperature programme based on the transition temperatures of the materials. Moreover, microscopy analysis of the materials during the recovery stage has also been reported (Lendlein and Langer, 2002a; Lendlein *et al.*, 2005b).

7.2.1 Stress–strain test

The stress–strain test is the procedure more commonly reported in the scientific literature to characterize the shape memory effect (Chen *et al.*, 2009; Liu *et al.*, 2007). It can be represented in a two-axis system, the variables of which are stress and strain for a fixed temperature. A more efficient representation of this test represents these variables in a three axis system, by adding the temperature axis. This will allow observing the temperature behaviour and locating the transition temperature. Figure 7.5 shows a typical stress–strain test in a three axis system. The complete shape memory test is constituted by a four-step cycle:



7.5 Cyclic stress–strain test.

- First of all, the test begins by heating the polymer, without applied stress, to a temperature above T_{trans} of the polymer.
- The second step is to apply a stress; it produces a strain in the material (ε_m).
- The stress is held while the material is cooled to a temperature below the transition temperature. Once the temporary shape is fixed (ε_m), the stress is removed.
- Finally the polymer is reheated above its transition temperature and in this way the polymer recovers its original shape (ε_p).

This procedure can be cyclically repeated with the aim of studying changes in shape memory properties. The main parameters used to quantify the shape memory effect are:

- *Strain recovery:* the ability of the material to return to its original shape.

$$R_r = \frac{\varepsilon_m - \varepsilon_p}{\varepsilon_m} \qquad [7.1]$$

- *Strain fixity:* representing the capability of material to keep its temporary shape when it has been deformed and cooled and the stress released.

$$R_f = \frac{\varepsilon_u}{\varepsilon_m} \qquad [7.2]$$

7.2.2 Bending test

Bending tests associated with thermal cycles are also able to characterize the shape memory effect in polymers (Knight *et al*., 2009; Luo *et al*., 2008; Zheng *et al*., 2006, 2008; Zhou *et al*., 2007). In the flexure test the measured quantity is the angle of deformation. The following coefficient is defined to quantify the ability of a material to recover its original shape:

$$R_b = \frac{\theta_i - \theta_f}{\theta_i} \qquad [7.3]$$

Another important parameter in SMPs is the recovery time. There is not agreement in the literature in quantifying this parameter; some authors have used a strain recovery versus time graph (Knight *et al*., 2009; Song *et al*., 2010b), others show photographs (Liu *et al*., 2002; Zhang *et al*., 2007; Zini *et al*., 2007) and others give a value of the recovery process (Luo *et al*. 2008; Zini *et al*., 2007).

7.3 Classifying shape memory polymers: classification by polymer structure

Different classifications have been reported for SMPs. Here, we report the main two classifications of these materials. The first one (discussed in this section) is based on the structure of the polymer matrix and the second one (discussed in the following section) depends on the external stimulus that provides the shape memory change. Shape memory behaviour is not an intrinsic property of the materials but it is necessary that polymers present an adequate morphology in order to show this behaviour. The necessity of fixing the shape of the polymer can be achieved through the generation of strong interactions among the polymer chains. In the next classification, four different polymer structures will be summarized depending on the cross-linking characteristics: chemically cross-linked, physically cross-linked, polymer blends and polymer composites.

7.3.1 Chemically cross-linked polymers

These SMPs are polymer networks formed by polymer chains cross-linked with covalent bonds. The network can be generated during polymerization or in a post-curing process. The covalent bonds are the fixity phase and the polymer chains are the switch phase. When the covalent bonds are introduced in the polymer matrix, the fixed shape of this polymer cannot be further changed. Thus, the polymeric matrix allows formation of the temporary shape and the recovery can be activated through the cross-links. Moreover, depending on the nature of the chains, the transition temperature might be the glass transition temperature (T_g) (Liu *et al*., 2002; Song *et al*., 2010b) or the melt temperature (T_m) (Lendlein *et al*., 2001; Nagata and Yamamoto, 2009). The main difference between T_g and T_m is that the T_g presents a wide interval of temperatures while T_m has a narrow interval (Behl *et al*., 2010) making it preferable to use T_m as the switch temperature.

There are several ways to generate cross-links in the matrix in order to obtain a SMP (Choi and Lendlein, 2007; Choi *et al*., 2006; Helminen *et al*., 2002; Kelch *et al*., 2007; Liu *et al*., 2002; Morshedian *et al*., 2003; Nagata and Kitazima, 2006; Nagata and Yamamoto, 2009; Nair *et al*., 2010; Schoener *et al*., 2010; Wischke *et al*., 2010; Zhu *et al*., 2006). They can be mainly grouped as two types: cross-linked by means of a cross-linking agent and cross-linking through electromagnetic radiation. For the first case, by adding peroxide to the polymer matrix it is possible to create covalent bonds (Helminen *et al*., 2002; Li *et al*., 1999; Liu *et al*., 2002; Morshedian *et al*., 2003). Peroxide is a chemical cross-linker that is blended with the polymer at ambient temperature. When the temperature of the compound exceeds the curing temperature, peroxide begins to decompose causing the cross-linking of the polymer chains.

Regarding the second case, there are different wavelengths which can elicit polymer cross-linking with electromagnetic radiation. As stated before, the first polymer with shape memory behaviour was obtained by radiation of polyethylene with gamma rays (Liu *et al*., 2007). This high energetic radiation has been used to generate shape memory in other polymers like poly(ε-caprolactone) (PCL) (Zhu *et al*., 2003) and in a blend of PCL and poly(methyl vinyl siloxane) (PMVS) (Zhu *et al*., 2006).

Further, UV radiation has also been used in order to create a SMP. In this case it is necessary to incorporate photo-initiators or light-sensitive monomers to produce a polymer network. Knight and co-workers (2009) synthesized a covalent network from poly(lactide-co-glycolide) (PLGA) with polyhedral oligomeric silsesquioxanes (POSS) using tetra-thiol as cross-link agent and 2,2-dimethoxy-2-phenylacetophenone (DMPA) as radical photo-initiator. In this case the cross-linking begins with exposure of polymer to a wavelength radiation of 365 nm. Recently, reactions of thiol-ene have been

investigated to obtain SMPs with good strain fixity and a small time recovery (Nair *et al.*, 2010).

Another way to obtain a cross-linked network with UV radiation consists of the addition of functional monomers to polymers chains. These functional monomers have the ability to create covalent bonds when they are exposed to UV light. For instance, the addition of acrylic groups to polymer chains has been used to create SMPs for applications in the biomedical sector. This technique is interesting because it is easy to control the initial shape of the polymer and the degree of cross-linking. In this case, the network points are formed by photo-curing (Choi and Lendlein, 2007; Choi *et al.*, 2006; Helminen *et al.*, 2002; Kelch *et al.*, 2007; Schoener *et al.*, 2010; Song *et al.*, 2010a; Wischke *et al.*, 2010).

Nagata (2006, 2009, 2010) has developed SMPs from PCL, poly(ethylene glycol) (PEG) and poly(lactic acid) (PLA) using two different chain extenders. The first is acid 4,4´-(adipoyldioxy) dicinnamic (CAC) (Nagata and Kitazima, 2006) and the second is obtained from the condensation of 5-hydroxyisophthalic acid and 7-chlorocarbonylmethoxycumarin (ICM) (Nagata and Yamamoto, 2009, 2010). These chain extenders form a dimer, cyclobutane, when they are exposed to UV radiation with wavelengths above 280 nm. The dimerization can be broken by applying UV radiation at 254 nm wavelength. With the same approach, Lendlein has obtained a SMP with reversible light cross-linking by means of addition of acid cinnamic molecules (Lendlein *et al.*, 2005a). Through this reversible light cross-linking it is possible to obtain a SMP controlled by light instead of temperature, as reported in the following section.

Liquid crystalline networks

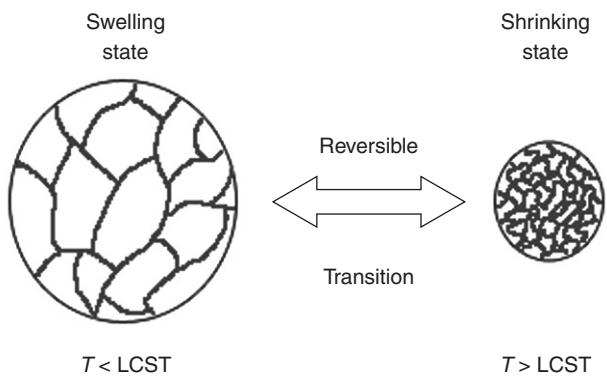
In general, liquid crystal is an aggregation state of the matter, which has a structural order like crystalline solids and at the same time can flow like liquid. Therefore, liquid crystal is an intermediate state of matter. The molecules that form liquid crystals are called mesogens. Liquid crystals can form different phases depending on mesogen orientation. Liquid crystal elastomers are cross-linked polymers with mesogens in the polymer backbone. Mesogens can be grafted in the main chain (Burke and Mather, 2010) or grafted in the lateral groups (Thomsen *et al.*, 2001). The cross-linking in the polymer chains prevents the flow of the polymer while the mesogens can be oriented by the phase transitions. Usually the nature of the cross-link is chemical (Burke and Mather, 2010; Jeong *et al.*, 2000a; Thomsen *et al.*, 2001), although some liquid crystal elastomers with physical cross-links have been also reported in the scientific literature (Jeong *et al.*, 2000b). In these polymers, the shape memory effect is reached by means of change in the mesogen structure when they undergo phase transformations (Burke

and Mather, 2010). These kinds of materials can be used as SMPs (Ahn *et al*., 2008), artificial muscle elements (Thomsen *et al*., 2001) and actuators (Liu *et al*., 2006).

Hydrogels

Hydrogels are three dimensional polymer networks. They are formed by polymer chains, which are cross-linked by chemical or physical bonds and the network can be swelled by water (Ahn *et al*., 2008). In fact, the hydrogel polymer chains are formed by hydrophilic monomers, able to absorb great amounts of water. Hydrogels have been studied widely in recent years, due to their ability to change their own structure in the presence of various stimuli, such as pH or temperature (Ahn *et al*., 2008). Temperature-sensitive gels have a solution critical temperature (SCT). There is a distinction in hydrogels according to their SCT: high solution critical temperature (HSCT) and low solution critical temperature (LSCT). HSCT hydrogels are swollen by the solvent when the temperature is above the HSCT and they release the solvent when temperature is below the HSCT. LSCT hydrogels exhibit the opposite behaviour: they are swollen when temperature is below the LSCT and they shrink if temperature exceeds LSCT. Normally this behaviour is reversible (Fig. 7.6) but can suffer aging under repeated cycles.

Osada (1995), Kagami (1996) and co-workers have been pioneers in the study of hydrogels as SMMs. Firstly they studied hydrogels from acrylic acid (AA) and stearyl acrylate (SA) copolymers chemically cross-linked with N,N´-methylene-bis(acryl amide) (MBAA) (Osada and Matsuda, 1995). Shape memory was obtained thanks to long hydrophobic regions from stearic acid contained in SA. These regions form a crystalline phase below 50°C and they act as a switching phase. This material has shape memory effect



7.6 Temperature effect in hydrogels.

when it is in the swollen state and the authors discovered that the transition temperature does not change by varying the co-monomer ratio in the copolymer. In further studies, the same authors replaced SA with methyl acrylate (MA) (Kagami *et al*., 1996). This hydrogel shows shape memory behaviour even in its shrunken state and the researchers demonstrated that it is possible to adjust the transition temperature by varying the amount of MA.

Poly(vinyl alcohol) (PVA) is a physically cross-linked hydrogel which shows shape memory effect (Hirai *et al*., 1992a, 1992b). The cross-linked network in this case is formed by hydrogen bonds. The principal drawback of PVA is its instability at temperatures above 60°C, due to the cleaving of hydrogen bonds. Thus adding chemical bonds can be a solution to overcome this drawback and, as reported in the literature, the chemical bonds can be provided by glutaraldehyde addition (Hirai *et al*., 1992a).

Another common hydrogel is polyacrylamide (PAAm). This hydrogel is used in drug delivery thanks to its low critical solution temperature which is close to body temperature. Moreover, exploiting this property, shape memory hydrogels can be obtained by a technique called modulated gel technology (Li *et al*., 1997). This technique consists of taking a substrate of PAAm and producing interpenetrating networks with *N*-isopropylacrylamide (NIPAAm) in some regions of the substrate in order to obtain the desired shape. When temperature increases NIPAAm shrinks and it produces shape changes. Additionally, by using this technology two-way SMPs can be developed (Li *et al*., 1997).

7.3.2 Physically cross-linked polymers

This class of SMP groups those polymers whose fixity phase is fixed by intermolecular forces such as crystalline phases (Cho *et al*., 2004; Jeong *et al*., 2000a), hydrogen bonds (Liu *et al*., 2000; Zhou *et al*., 2007) or ionic forces (Han *et al*., 2007; Kim *et al*., 1998; Zhu *et al*., 2006, 2008). The switch phase is provided by polymer chains through their own relaxation. Also in this case the transition temperature of the shape memory effect can be regulated by the T_m or by the T_g. A notable difference between physical and chemical cross-linking is that in the physically cross-linked polymers it is possible to remould the fixity phase. To obtain this effect, it is necessary to reach the temperature at which the physical bonds disappear allowing reshaping. In fact, when the polymer is cooled the physical bonds appear again. This process is not achieved in chemically cross-linked polymers because cleaving the covalent bonds leads to degradation of the polymer.

In general, polymers with amorphous and crystalline regions can themselves be candidates for SMMs. In this case, the fixity shape is established by the crystals and the switching phase is due to the amorphous chains.

Therefore some authors (Lu *et al*., 2007, 2008; Wong and Venkatraman, 2010) have studied shape memory properties in semi-crystalline polymers such as PLA. PLA is an interesting polymer because it is biodegradable and biocompatible and it shows relatively good mechanical properties (Knight *et al*., 2009; Lu *et al*., 2007, 2008; Meng *et al*., 2009; Min *et al*., 2005; Nagahama *et al*., 2009; Nagata and Yamamoto, 2010; Wang *et al*., 2006; Wong and Venkatraman, 2010). PLA also shows shape memory behaviour but it is limited to small stresses and in a certain temperature range. So, the strategy to improve the shape memory properties in these polymers consists of obtaining a phase separation morphology. This goal is reached with block copolymers, polyurethanes, blends, composite materials, etc.

Block copolymers

Through the block copolymerization of two different homopolymers it is possible to obtain a system with separated phases (Lu *et al*., 2008; Luo *et al*., 2000; Meng *et al*., 2009; Min *et al*., 2005). The co-monomer with the highest transition temperature acts as the fixity phase, while the other one is the switching phase. Also in this case the transition temperature can be T_g or T_m depending on the nature of the co-monomer. Moreover, the copolymerization allows improvement in other properties like biodegradability, hydrophilicity, solvent absorption and diffusion, toughness, transition temperature or activation shape memory time.

As mentioned above, PLA is a thermoplastic polymer with shape memory behaviour. This polymer can be used as co-monomer with the aim of improving its properties. For instance, copolymers from lactic acid with glycolic acid (i.e., poly(lactic-co-glycolic acid) (PLGA)) (Meng *et al*., 2009) or ε-caprolactone (to form poly(L-lactide-co-ε-caprolactone) (PCLA))(Lu *et al*., 2008) present better shape memory behaviour than the PLA homopolymer. Other copolymers like poly(ethylene oxide) (PEO) with poly(ethylene terephthalate) (PET) have been studied (Luo *et al*., 2000). In this copolymer PEO is the switching phase with transition temperature between 40°C and 50°C and PET is the fixity phase.

Thermoplastic polyurethanes

Synthesized polyurethanes (PU) from diols of thermoplastic polymers constitute good candidates for SMPs taking advantage of their phase separation (Cao and Liu, 2006; Kim *et al*., 1998; Lendlein *et al*., 2009; Nagahama *et al*., 2009; Wang *et al*., 2006; Xue *et al*., 2010; Zhu *et al*., 1998, 2006). The synthetic procedure is based on the prepolymer method. In the first step a thermoplastic polymeric diol reacts with a diisocyanate to form the prepolymer. This prepolymer reacts with low molecular weight diols called chain extenders.

Long polymer chains with urethane groups, highly polar segments, can be obtained with this method. These groups form the fixity phase by means of hydrogen bonds and dipole–dipole interactions. Switching segments are formed by the oligoester. This phase separation can be seen with atom force microscopy (Cao and Liu, 2006). PLA is again a good candidate to develop shape memory polyurethanes (Wang *et al.*, 2006). Moreover, other aliphatic polyesters such as PCL (Nagahama *et al.*, 2009) have been used as precursors in synthesis of shape memory polyurethanes. In this case the T_m of PCL acts as the switching phase.

Another way to obtain shape memory polyurethanes is by joining block copolymers (Lendlein *et al.*, 2009; Xue *et al.*, 2010) with urea bonds. Lendlein (2009) investigated different polyurethanes from copolymers formed by lactic acid with other monomers. He obtained SMPs with tuneable transition temperature between 14°C and 56°C, depending on the monomers used and on their concentrations. Kim (1998) and co-workers have synthesized PCL polyurethanes with ionomers, monomers joined to ionic centres. In this manner, cohesion in the fixity phase is improved thanks to the Coulombic interaction between ionomers, enhancing the mechanical properties. Y. Zhu (2006, 2008) and co-workers have performed an exhaustive study on the influence of ionomers. Moreover, ionomers have also been used to obtain SMPs with copolymers (Han *et al.*, 2007).

Polymer blends and IPNs

Blending is a good technique to obtain new polymeric materials and to enhance polymer properties such as thermal behaviour, mechanical properties, etc. Moreover, blending is an easy process to implement at the industrial level (Meng and Hu, 2009). For these reasons it is interesting to develop SMP blends. There are different methods to obtain polymer blends with shape memory behaviour that can be classified in two groups: miscible and immiscible blends.

Different morphologies able to produce shape memory miscible blends have been reported. For example, semi-crystalline and amorphous polymers can be blended as reported for PCL polyurethanes with phenolic resin (Jeong *et al.*, 2001a) or with poly(vinyl chloride) (PVC) (Jeong *et al.*, 2001b). Taking into account that blends of miscible polymers have only one T_g, it is possible to adjust the transition temperature varying the relative concentration of polymer components. Miscible blends of both semi-crystalline polymers have also been studied to develop SMPs, for instance, blends of polydioxianone and PCL (Behl *et al.*, 2009). The transition temperature in this polymer is the melting temperature of PCL. Recently H. Zhang and

co-workers reported SMP blends from immiscible polymers (Zhang *et al.*, 2009). These blends are constituted by a thermoplastic elastomer, poly (styrene-b-butadiene-b-styrene) (SBS) dispersed in a PCL matrix. Another immiscible blend with shape memory reported in the literature is formed by poly(L-lactide-co-ε-caprolactone) and PLGA (Liu *et al.*, 2005).

Another way to obtain SMP is based on the synthesis of interpenetrating polymer networks (IPNs) (Ahn *et al.*, 2008; Liu *et al.*, 2005; Zhang *et al.*, 2007). These materials are constituted by two miscible polymers whose networks are interlaced. The synthesis proceeds in three steps. In the first one both polymers are blended, after that both polymer networks are cross-linked in successive steps. S. Zhang and co-workers have synthesized IPNs from PEG dimethacrylates with PLGA polyurethanes (Zhang *et al.*, 2007). After blending the polymers, they radiated them with UV light. This radiation produced cross-linked PEG dimethacrylate IPNs by curing the acrylate groups. They obtained the second network by reaction of PLGA with an isocyanate. In this material the amorphous chains act as the switching phase while the covalent net points of both networks are the fixity phase. Miscibility implies one T_g for the IPN which is used as transition temperature and it can be modulated depending on the ratio of initial polymers. The authors show T_{trans} values from –24.9°C to 51.8°C with strain recovery and strain fixity over 93%.

G. Liu and co-workers have developed a two transition temperatures SMPs by synthesis of an IPN from PEO and poly(methyl methacrylate) (PMMA). When the concentration of PEO is over 30%, phase separation is obtained. One phase is formed by interpenetrating amorphous networks and the other is represented by the PEO crystals. Shape memory effect can occur through melt temperature of PEO crystals with IPN acting as fixity phase. It is also possible to obtain the shape memory effect through T_g of IPNs with fixity phase formed by the covalent bonds. The same authors have developed a shape memory IPN from poly(methyl methacrylate-*N*-vinyl-2-pyrrolidone) and PEG (Liu *et al.*, 2006) linked by hydrogen bonds.

Composites

There is a broad range of studies on the SMP composites. These materials are based on a polymeric matrix with fillers or nanofillers. Shape memory PLLA matrix composites with ceramic fillers, hydroxyapatite (Yu *et al.*, 2009; Zhou *et al.*, 2007) and β-tricalcium (Zheng *et al.*, 2008), have been reported with improved properties with respect to the PLLA homopolymer. In this case, PLLA chains are the switching phase while the fixity phase is constituted by the interaction between the particles and the polymer. Meng (2009) has achieved enhanced shape memory properties of PLLA using fillers of the biopolymer chitosan. In the following

section the addition of certain fillers allowing stimuli sensitive SMP will be discussed.

7.4 Classifying shape memory polymers: classification by type of stimulus

In the following section the main stimuli able to produce shape memory effects in polymeric materials are discussed. A general classification in direct and non-direct stimuli is proposed (Meng and Hu, 2009). Direct stimuli allow programming and recovery stages while non-direct stimuli act only during the recovery process making use of another stimulus necessary for the temporary fixation. In fact, for non-direct stimuli, the programming phase is performed through temperature changes, using a similar temperature-sensitive programming procedure as discussed above. Indirect stimuli are typically moisture, electric and magnetic fields. Light can act as both direct or indirect stimulus.

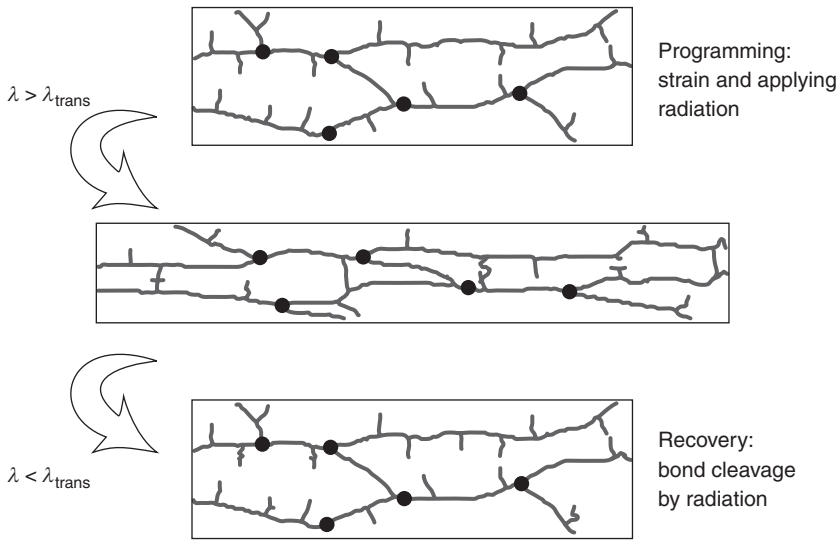
7.4.1 Stimulation by light radiation

As indicated by Jiang and co-workers (2006) in their review, there are three kinds of photo-responsive molecules: photoisomerizables (azobenzenes) (Ikeda *et al*., 2003; Zeng *et al*., 2010), molecules obtained by cationic induced polymerization (Irie and Kunwatchakun, 1986) and photo-responsive molecules (i.e. cinnamic acid). Photoisomerizable molecules possess the ability to change their configuration from *trans* to *cis* and vice versa, when they are exposed to UV light. This transition causes a stress and consequently a shape change in the polymer.

In Fig. 7.7 it is possible to see the configuration capable of producing shape memory effect with photo-reactive molecules (light grey triangles). These molecules are the switch phase and the covalent bonds between polymer chains (black dots) are the fixity phase. In the presence of electromagnetic radiation ($\lambda > \lambda_{trans}$), the photo-reactive molecules form dimers (grey diamonds) that are responsible to fix the temporary shape. The polymer recovers its original shape when it is exposed to another radiation ($\lambda < \lambda_{trans}$).

Lendlein and co-workers (2005a) synthesized two different configurations with cinnamic acid in the matrix, grafting these molecules and producing an interpenetrating polymer network. By this method they obtained excellent values for strain recovery, around 98%. However, the strain fixity had small values, 52% in the best case.

Light activation has a significant advantage in comparison with the thermal shape memory behaviour, as photo-activation does not produce tissue damage as could be produced by heat treatments. This fact makes light activation attractive



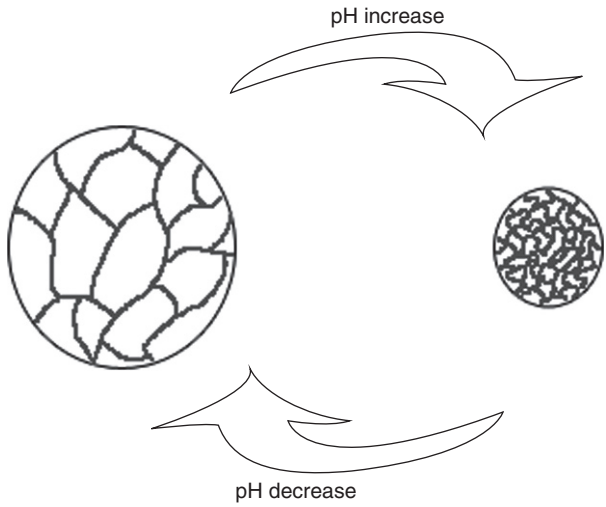
7.7 Directly light-activated shape memory mechanism.

for biomedical applications (Jiang *et al.*, 2006). It has been also reported that infrared light can be used to produce indirect shape memory effect on composites based on a polymer matrix reinforced by carbon black (CB) or carbon nanotubes (CNTs) (Wong and Venkatraman, 2010). These nanofillers enhance the thermal conductivity of the polymer. The programming stage is analogous to the thermal induced shape memory mechanism. But in this case, the recovery occurs when the polymer is irradiated with infrared light.

7.4.2 Stimulation by pH changes

The most common pH-sensitive polymers are the hydrogels. As mentioned above, polymer gels are able to swell in the presence of a solvent and they can keep large amounts of solvent within them. This particular behaviour is controlled by temperature (Lee *et al.*, 1996): the hydrogel collapses when the temperature is above the HCST or below the LCST and this fact produces the solvent expulsion and gel shrinkage (Fig. 7.6). In addition to thermosensitive hydrogels, there are also pH-sensitive hydrogels. These materials are achieved by adding active groups in the gel structure which can be positively or negatively ionized (Ahn *et al.*, 2008; Gil and Hudson, 2004; Jagur-Grodzinski, 2010).

There are two ionic groups available: polyacids and polybases. The first ones accept protons at low pH, producing gel shrinkage. Conversely,



7.8 Swelling–shrinkage behaviour in ionic based hydrogel by changes in pH.

polybases donate protons at high pH (Fig. 7.8). Gel swelling or shrinkage results from the Coulombic interactions of the ionic groups and the associated change in the osmotic pressure. This process is reversible if the gel is immersed in its own solvent. Moreover, the degree of swelling can also be controlled by changing chain hydrophobicity (Markland *et al*., 1999). The main applications of hydrogels are in the biomedical and pharmaceutical fields; in fact they are commonly used as devices for drug delivery.

7.4.3 Stimulation by moisture

This peculiar effect was discovered in 2003 by Yang and co-workers (2004). They studied a shape memory polyurethane in temporary shape with a T_g equal to 35°C and observed a recovery of the original shape at room temperature in a month. They characterized this polyurethane and they concluded that moisture was the cause of the recovery process. In this case the programming stage is analogous to the thermally activated one: the polymer is heated above the transition temperature, in this case the glass transition temperature, so that the temporal shape is fixed and then the polymer is cooled. The recovery mechanism is controlled by moisture gradually being absorbed by the polymer. The water acts as a plasticizer, weakening the hydrogen bonds and producing a decrease in its transition temperature. Thus, the chains become more flexible making possible recovery of the initial shape at room temperature. For example, the recovery time of PVA

immersed in different solvents has been studied and the best recovery time was 45 min for water immersion (Du and Zhang, 2010).

Analogous to light-induced effects, moisture sensitive shape polymers are interesting materials to use as smart materials at constant temperatures because it is not necessary to apply heat to obtain shape transition. In this manner they can be used in biological systems where the heat can produce tissue damage (Du and Zhang, 2010; Huang *et al.*, 2010).

7.4.4 Stimulation by electric field

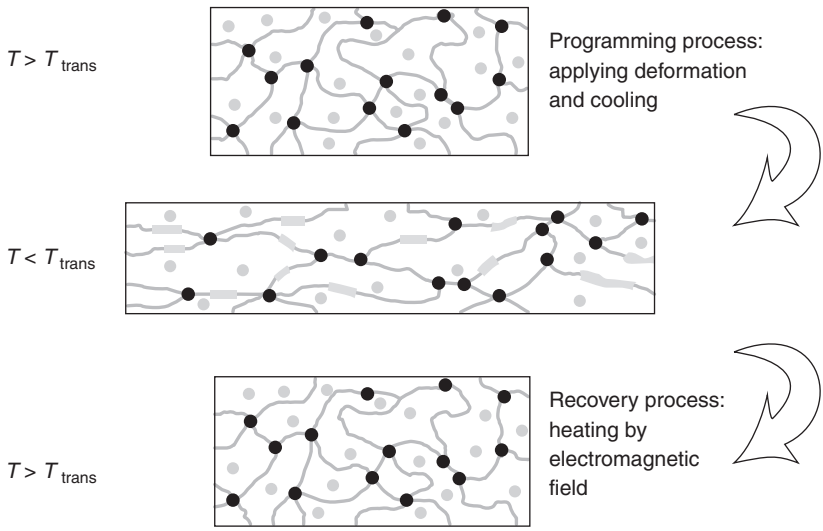
Electric field sensitive polymers can be achieved by adding conductive fillers to the polymeric matrix. In fact, these fillers can transform the electric energy into thermal energy when they are immersed in an electric field (Liu *et al.*, 2009; Meng and Hu, 2009). In particular, different types of carbon nanoparticles such as carbon nanotubes (CNTs), carbon fibers (CFs) or carbon black (CB) have been studied to obtain electro-sensitive SMPs (Cho *et al.*, 2005; Gunes *et al.*, 2009; Jung *et al.*, 2010; Leng *et al.*, 2007). In this case, the energy transformation is due to the Joule effect and when the polymer reaches its transition temperature the recovery process can start.

The change in electric conductivity by varying the applied stress, the filler type and concentration and the temperature of materials has been extensively reported (Gunes *et al.*, 2009; Leng *et al.*, 2007). Cho (2005) investigated shape memory polyurethanes with multiwall CNTs. The author evidenced two important results: the enhancement of mechanical properties of the nanocomposite with respect to the neat matrix and its electro-sensitive response, making it a good candidate for an electrical actuator.

7.4.5 Stimulation by magnetic field

Magnetically induced shape memory effects are achieved by means of nanocomposites loaded with specific magnetic nanoparticles. The magnetically activated shape memory process is represented in Fig. 7.9. The nanofillers (light grey circles in Fig. 7.9) are superparamagnetic and they produce heat when they are immersed in a magnetic field (Mohr *et al.*, 2006; Schmidt, 2006; Zheng *et al.*, 2009). Their temporary shape is fixed in the same manner as that in the thermally induced one. Recovery is produced by the Joule effect when an external magnetic field is applied.

The fillers can be iron oxides such as magnetite (Fe_3O_4) (Schmidt, 2006; Zheng *et al.*, 2009) or Fe(III) oxide (Schmidt, 2006) or other superparamagnetic particles (Fuhrer *et al.*, 2009). For instance, magnetite has been added to PLA, PCL and poly(butyl acrylate) (PBA) matrixes (Schmidt, 2006; Zheng *et al.*, 2009). This oxide has the advantage of being a biocompatible material,



7.9 Magnetically activated shape memory process.

thus allowing its use in biomedical applications. Magnetosensitive particles have also been implanted in shape memory hydrogels (Fuhrer *et al.*, 2009).

7.5 Main applications of smart polymers

Shape memory materials are useful for many applications and interest in them is likely to increase in the future. In this section we report the main applications of SMPs by dividing them into two sub-sections: the first one is dedicated to biomedical applications as this sector is the most active in current research and industrial developments while the second one groups all the other applications. The main applications of SMPs reported in the scientific literature are summarized in Table 7.2.

7.5.1 Biomedical applications: biocompatible shape memory polymers

The use of polymers in the biomedical sector began in the mid-twentieth century and, since then, has increased constantly. It is important to emphasize that there are specific requirements and limitations in using SMPs for biomedical applications. They should have small recovery times, be biocompatible and, depending on their specific applications, should be also biodegradable. Moreover, for these SMMs, their transition temperature should be in the order of body temperature in order to avoid tissue harm and to be able to use body temperature as the switching temperature.

Table 7.2 Main applications of SMPs in literature

Application	Author
Sutures	Lendlein and Langer, 2002a
Vascular stents	Kim *et al.*, 2010; Peng *et al.*, 1996; Xue *et al.*, 2010
Microactuator to prevent ischaemic stroke	Maitland *et al.*, 2002; Metzger *et al.*, 2002; Small *et al.*, 2005
Tissue engineering scaffolds	Migneco *et al.*, 2009; Neuss *et al.*, 2009
Ophthalmologic devices	Song *et al.*, 2010a, 2010b
Self-healing materials	Nji and Li, 2010
Drug delivery systems	Wischke and Lendlein, 2010; Wischke *et al.*, 2010
Shrinkable tubes	Morshedian *et al.*, 2003; Ota, 1981
Aerospace devices	Arzberger *et al.*, 2005; Loughlan *et al.*, 2002
Micro electro mechanical systems (MEMS)	Gall *et al.*, 2002, 2004
Actuators	Cho *et al.*, 2004; Fuhrer *et al.*, 2009; Koerner *et al.*, 2004; Takashima *et al.*, 2010; Vaia, 2005

Aliphatic polyesters such as PLA, PCL or polydioxanone (PDO) have seen extensive use in medicine because they are both biocompatible and biodegradable. As mentioned above, PLA homopolymer has shape memory itself (Lu *et al.*, 2007, 2008; Wong *et al.*, 2008). In order to enhance its shape memory behaviour, copolymers, polyurethanes and composites based on PLA have been developed. PCL is a semi-crystalline polymer and its melting point is near body temperature (Nagahama *et al.*, 2000). For this reason PCL is widely used as switch phase. Recently, a study on PCL copolymerized with glycolic acid has been reported (Min *et al.*, 2005). Lendlein and Langer (2002) developed suture wires for tissue repair from polyesters PCL and PDO taking advantage of their biodegradability.

One of the most studied applications is the vascular stent to prevent vessel obstruction (Kim *et al.*, 2010; Peng *et al.*, 1996; Xue *et al.*, 2010). Usually they are based on Nitinol; however, the advantages given by SMPs, such as biodegradability and low cost, have led to an increasing interest in using these materials. Tamai *et al.* (2000) reported the first study of the effects of these stents implanted over six months in the human body. Micro-actuators based on SMPs to prevent ischaemic stroke have also been developed (Maitland *et al.*, 2002; Metzger *et al.*, 2002; Small *et al.*, 2005). Small and co-workers (2005) reported that it is possible to create silicon actuators loaded with CNTs activated by infrared light.

Besides suture wires and vascular stents, other biomedical applications with SMPs include scaffolds for tissue engineering (Migneco *et al.*, 2009; Neuss *et al.*, 2009), ophthalmologic materials (Song *et al.*, 2010a, b),

self-healing materials for biomimetic devices (Nji and Li, 2010) and drug delivery systems (Wischke and Lendlein, 2010; Wischke *et al*., 2009).

7.5.2 Other applications

Besides biomedical applications there are many applications of SMPs in other technological sectors. For instance, one of the first applications of SMPs was in shrinkable tubes produced with irradiated, cross-linked, low-density polyethylene for the packaging industry. (Morshedian *et al*., 2003; Ota, 1981). Applications in the aerospace industry have also been studied (Arzberger *et al*., 2005; Loughlan *et al*., 2002), although extensive applications in high technology industries are limited, particularly due to the poor mechanical properties of polymers compared to metallic materials. With the aim of improving these properties, many authors have reported studies on polymer composites with shape memory reinforced with carbon nanotubes (Gall *et al*., 2002, 2004; Meng and Hu, 2009). Moreover, by working with polymer composites it is possible to trigger the shape recovery through different stimuli as seen in this review. These features make SMPs great candidates for use as the active component in sensors and/or actuators (Cho *et al*., 2004; Fuhrer *et al*., 2009; Koerner *et al*., 2004; Takashima *et al*., 2010; Vaia, 2005). Gall *et al*. (2002, 2004) studied the use of SMPs as 'micro electrical mechanical systems' (MEMS), micro-transducers and micro-valves, reporting that it is possible to control the flow in a polymer tube through the application of heat.

7.6 Conclusion

In conclusion, SMP materials are one of the most dynamic sectors of research in materials science and technology providing excellent opportunities for scientific developments in the field of molecular design, polymer synthesis and functionalization, viscoelastic and electrical properties and the processing of polymer blends, copolymers, composites and nanocomposites. Amazing technological developments and applications can be foreseen in future years in high technology sectors, starting with the biomedical sector and followed by advanced mechanical sectors, mechatronic and power generation among others. However, great research efforts are required to obtain controlled response materials with durability and reliability over those offered by emerging technologies currently available in this sector.

7.7 References

Ahir, S.V. and E.M. Terentjev (2005), Photomechanical actuation in polymer-nanotube composites. *Nature Materials*, **4**(6): pp. 491–495.

Ahn, S.K., R.M. Kasi, S.C. Kim, N. Sharma and Y. Zhou (2008), Stimuli-responsive polymer gels. *Soft Matter*, **4**(6): pp. 1151–1157.

Arzberger, S.C., M.L Tupper, M.S. Lake, R. Barrett, K. Mallick, C. Hazelton, W. Francis, P.N. Keller, D. Campbell, S. Feucht, D. Codell, J. Wintergerst, L. Adams, J. Mallioux, R. Denis, K. White, M. Long, N.A. Munshi and K. (2005), *Elastic Memory Composites (EMC) for deployable industrial and commercial applications.* Smart Structures and Materials 2005: Industrial and Commercial Applications of Smart Structures Technologies, **5762**: pp. 35–47.

Behl, M., U. Ridder, Y. Feng, S. Kelch and A Lendlein (2009), Shape-memory capability of binary multiblock copolymer blends with hard and switching domains provided by different components. *Soft Matter*, **5**(3): pp. 676–684.

Behl, M., M.Y. Razzaq and A. Lendlein (2010), Multifunctional shape-memory polymers. *Advanced Materials*, **22**(31): pp. 3388–3410.

Bigelow, G.S., S.A. Padula, A. Garg, D. Gaydosh and R.D. Noebe (2010), Characterization of ternary NiTiPd high-temperature shape-memory alloys under load-biased thermal cycling. *Metallurgical and Materials Transactions a-Physical Metallurgy and Materials Science*, **41A**(12): pp. 3065–3079.

Buehler, W.J., R.C. Wiley and J.V. Gilfrich (1963), Effect of low-temperature phase changes on mechanical properties of alloys near composition TiNi. *Journal of Applied Physics*, **34**(5): pp. 1475–1477.

Burke, K.A. and P.T. Mather (2010), Soft shape memory in main-chain liquid crystalline elastomers. *Journal of Materials Chemistry*, **20**(17): pp. 3449–3457.

Cao, Q. and P.S. Liu (2006), Structure and mechanical properties of shape memory polyurethane based on hyperbranched polyesters. *Polymer Bulletin*, **57**(6): pp. 889–899.

Chang, C.Y., D. Vokoun and C.T. Hu (2001), Two-way shape memory effect of NiTi alloy induced by constraint aging treatment at room temperature. *Metallurgical and Materials Transactions a-Physical Metallurgy and Materials Science*, **32**(7): pp. 1629–1634.

Chang, L.C. and T.A. Read (1951), Plastic deformation and diffusionless phase changes in metals – the gold-cadmium beta-phase. *Transactions of the American Institute of Mining and Metallurgical Engineers*, **191**(1): pp. 47–52.

Chau, E.T.F., C.M. Friend, D.M. Allen, J. Hora and J.R. Webster (2006), A technical and economic appraisal of shape memory alloys for aerospace applications. *Materials Science and Engineering a-Structural Materials Properties Microstructure and Processing*, **438**: pp. 589–592.

Chen, S., J. Hu, H. Zhuo and Y. Zhu (2008), Two-way shape memory effect in polymer laminates. *Materials Letters*, **62**(25): pp. 4088–4090.

Chen, S.J., J.L. Hu and H.T. Zhuo (2010), Properties and mechanism of two-way shape memory polyurethane composites. *Composites Science and Technology*, **70**(10): pp. 1437–1443.

Chen, X.H., J. Wang, J. Zou, X. Wu, X. Chen and F. Xue (2009), Mechanical and thermal properties of functionalized multiwalled carbon nanotubes and multiwalled carbon nanotube-polyurethane composites. *Journal of Applied Polymer Science*, **114**(6): pp. 3407–3413.

Cho, J.W., J.W. Kim, Y.C. Jung and N.S. Goo (2005), Electroactive shape-memory polyurethane composites incorporating carbon nanotubes. *Macromolecular Rapid Communications*, **26**(5): pp. 412–416.

Cho, J.W., Y.C. Jung, Y.C. Chung and B.C. Chun (2004), Improved mechanical properties of shape-memory polyurethane block copolymers through the control of the soft-segment arrangement. *Journal of Applied Polymer Science*, **93**(5): pp. 2410–2415.

Choi, N.Y. and A. Lendlein (2007), Degradable shape-memory polymer networks from oligo (L-lactide)-ran-glycolide dimethacrylates. *Soft Matter*, **3**(7): pp. 901–909.

Choi, N.Y., S. Kelch and A. Lendlein (2006), Synthesis, shape-memory functionality and hydrolytical degradation studies on polymer networks from poly(rac-lactide)b-poly(propylene oxide)-b-poly(rac-lactide) dimethacrylates. *Advanced Engineering Materials*, **8**(5): pp. 439–445.

Colic, M., R. Rudolf, D. Stamenkovic, I. Anzel, D. Vucevic, M. Jenko, V. Lazic and G. Lojen (2010), Relationship between microstructure, cytotoxicity and corrosion properties of a Cu-Al-Ni shape memory alloy. *Acta Biomaterialia*, **6**(1): pp. 308–317.

Du, H.Y. and J.H. Zhang (2010), Solvent induced shape recovery of shape memory polymer based on chemically cross-linked poly(vinyl alcohol). *Soft Matter*, **6**(14): pp. 3370–3376.

Fuhrer, R., E.K. Athanassiou, N.A. Luechinger and W.J. Stark (2009), Crosslinking metal nanoparticles into the polymer backbone of hydrogels enables preparation of soft, magnetic field-driven actuators with muscle-like flexibility. *Small*, **5**(3): pp. 383–388.

Gall, K., M. Mikulas, N.A. Munshi, F. Beavers and M. Tupper (2000), Carbon fiber reinforced shape memory polymer composites. *Journal of Intelligent Material Systems and Structures*, **11**(11): pp. 877–886.

Gall, K., M.L.Dunn, Y. Liu, D. Finch, M. Lake and N.A. Munshi (2002), Shape memory polymer nanocomposites. *Acta Materialia*, **50**(20): pp. 5115–5126.

Gall, K., P. Kreiner, D. Turner and M. Hulse (2004), Shape-memory polymers for microelectromechanical systems. *Journal of Microelectromechanical Systems*, **13**(3): pp. 472–483.

Gil, E.S. and S.M. Hudson (2004), Stimuli-responsive polymers and their bioconjugates. *Progress in Polymer Science*, **29**(12): pp. 1173–1222.

Gil, F.J., E. Solano, E. Engel, A. Mendoza and J.A. Planell (2004), Microstructural, mechanical and cytotoxicity evaluation of different NiTi and NiTiCu shape memory alloys. *Journal of Materials Science-Materials in Medicine*, **15**(11): pp. 1181–1185.

Gunes, I.S., G.A. Jimenez and S.C. Jana (2009), Carbonaceous fillers for shape memory actuation of polyurethane composites by resistive heating. *Carbon*, **47**(4): pp. 981–997.

Han, S.I., B.H. Gu, K.H. Nam, S.J. Im, S.C. Kim and S.S. Im (2007), Novel copolyester-based ionomer for a shape-memory biodegradable material. *Polymer*, **48**(7): pp. 1830–1834.

Hane, K.F. and T.W. Shield (1999), Microstructure in a copper-aluminium-nickel shape-memory alloy. *Proceedings of the Royal Society a-Mathematical Physical and Engineering Sciences*, **455**(1991): pp. 3901–3915.

He, X.M., L.J. Rong, D.S. Yan and Y.Y. Li (2005), Temperature memory effect of $Ni_{47}Ti_{44}Nb_9$ wide hysteresis shape memory alloy. *Scripta Materialia*, **53**(12): pp. 1411–1415.

Helminen, A.O., H. Korhonen and J.V. Seppala (2002), Cross-linked poly(epsilon-caprolactone/D,L-lactide) copolymers with elastic properties. *Macromolecular Chemistry and Physics*, **203**(18): pp. 2630–2639.

Heuer, A.H., M. Ruhle and D.B. Marshall (1990), On the thermoelastic martensitic-transformation in tetragonal zirconia. *Journal of the American Ceramic Society*, **73**(4): pp. 1084–1093.

Hirai, T., H. Matuyama, T. Suzuki and S. Hayashi (1992a), Effect of chemical cross-linking under elongation on shape restoring of poly(vinyl alcohol) hydrogel. *Journal of Applied Polymer Science*, **46**(8): pp. 1449–1451.

Hirai, T., H. Matuyama, T. Suzuki and S. Hayashi (1992b), Shape memorizing properties of a hydrogel of poly(vinyl alcohol). *Journal of Applied Polymer Science*, **45**(10): pp. 1849–1855.

Hornbogen, E. (2006), Comparison of shape memory metals and polymers. *Advanced Engineering Materials*, **8**(1–2): pp. 101–106.

Huang, W.M., B. Yang, Y. Zhao and Z. Ding (2010), Thermo-moisture responsive polyurethane shape-memory polymer and composites: a review. *Journal of Materials Chemistry*, **20**(17): pp. 3367–3381.

Ikeda, T., M. Nakano, Y. Yu, O. Tsutsumi and A. Kanazawa (2003), Anisotropic bending and unbending behavior of azobenzene liquid-crystalline gels by light exposure. *Advanced Materials*, **15**(3): pp. 201–205.

Irie, M. and D. Kunwatchakun (1986), Photoresponsive polymers.8. Reversible photostimulated dilation of polyacrylamide gels having triphenylmethane leuco derivatives. *Macromolecules*, **19**(10): pp. 2476–2480.

Jagur-Grodzinski, J. (2010), Polymeric gels and hydrogels for biomedical and pharmaceutical applications. *Polymers for Advanced Technologies*, **21**(1): pp. 27–47.

Jeong, H.M., B.K. Ahn and B.K. Kim (2001a), Miscibility and shape memory effect of thermoplastic polyurethane blends with phenoxy resin. *European Polymer Journal*, **37**(11): pp. 2245–2252.

Jeong, H.M., B.K. Kim and Y.J. Choi (2000a), Synthesis and properties of thermotropic liquid crystalline polyurethane elastomers. *Polymer*, **41**(5): pp. 1849–1855.

Jeong, H.M., J. H. Song (2001b), Miscibility and shape memory property of poly(vinyl chloride)/thermoplastic polyurethane blends. *Journal of Materials Science*, **36**(22): pp. 5457–5463.

Jeong, H.M., J.B.Lee, S.Y. Lee and B.K. Kim (2000b), Shape memory polyurethane containing mesogenic moiety. *Journal of Materials Science*, **35**(2): pp. 279–283.

Jiang, H.Y., S. Kelch and A. Lendlein (2006), Polymers move in response to light. *Advanced Materials*, **18**(11): pp. 1471–1475.

Jung, Y.C., H.H. Kim, Y.A. Kim, J.H. Kim, J.W. Cho, M. Endo and M.S. Dresselhaus (2010), Optically active multi-walled carbon nanotubes for transparent, conductive memory-shape polyurethane film. *Macromolecules*, **43**(14): pp. 6106–6112.

Kagami, Y., J.P. Gong and Y. Osada (1996), Shape memory behaviors of cross-linked copolymers containing stearyl acrylate. *Macromolecular Rapid Communications*, **17**(8): pp. 539–543.

Karp, J.M. and R. Langer (2007), Development and therapeutic applications of advanced biomaterials. *Current Opinion in Biotechnology*, **18**(5): pp. 454–459.

Kelch, S., S. Steuer, A.M. Schmidt and A. Lendlein (2007), Shape-memory polymer networks from oligo (epsilon-hydroxycaproate)-co-glycolate dimethacrylates and

butyl acrylate with adjustable hydrolytic degradation rate. *Biomacromolecules*, **8**(3): pp. 1018–1027.

Kim, B.K., S.Y. Lee, J.S. Lee, S.H. Baek, Y.J. Choi, J.O. Lee and M. Xu (1998), Polyurethane ionomers having shape memory effects. *Polymer*, **39**(13): pp. 2803–2808.

Kim, J.H., T.J. Kang and W.R. Yu (2010), Simulation of mechanical behavior of temperature-responsive braided stents made of shape memory polyurethanes. *Journal of Biomechanics*, **43**(4): pp. 632–643.

Knight, P.T., K.M. Lee, T. Chung, P.T. Mather (2009), PLGA-POSS end-linked networks with tailored degradation and shape memory behavior. *Macromolecules*, **42**(17): pp. 6596–6605.

Koerner, H., G. Price, N.A. Pearce, M. Alexander and R.A. Vaia (2004), Remotely actuated polymer nanocomposites – stress-recovery of carbon-nanotube-filled thermoplastic elastomers. *Nature Materials*, **3**(2): pp. 115–120.

Lee, Y.M., S.H. Kim and C.S. Cho (1996), Synthesis and swelling characteristics of pH and thermoresponsive interpenetrating polymer network hydrogel composed of poly(vinyl alcohol) and poly(acrylic acid). *Journal of Applied Polymer Science*, **62**(2): pp. 301–311.

Lendlein, A. and R. Langer (2002a), Biodegradable, elastic shape-memory polymers for potential biomedical applications. *Science*, **296**(5573): pp. 1673–1676.

Lendlein, A. and S. Kelch (2002b), Shape-memory polymers. *Angewandte Chemie-International Edition*, **41**(12): pp. 2034–2057.

Lendlein, A., J. Canisius, J. Schulte and K. Kratz (2004), Shape memory polymers as stimuli-sensitive implant materials for medical applications. SMST-2003: Proceedings of the International Conference on Shape Memory and Superelastic Technologies, ed. A.R. Pelton and T. Duerig. 563–572.

Lendlein, A., H. Jiang, O. Jünger and R. Langer (2005a), Light-induced shape-memory polymers. *Nature*, **434**(7035): pp. 879–882.

Lendlein, A., A.M. Schmidt and R. Langer (2001), AB-polymer networks based on oligo(epsilon-caprolactone) segments showing shape-memory properties. *Proceedings of the National Academy of Sciences of the United States of America*, **98**(3): pp. 842–847.

Lendlein, A., A.M. Schimdt, M. Schroeter and R. Langer (2005b), Shape-memory polymer networks from oligo(epsilon-caprolactone)dimethacrylates. *Journal of Polymer Science Part a-Polymer Chemistry*, **43**(7): pp. 1369–1381.

Lendlein, A., J. Zotzmann, Y. Feng, A. Altheld and S. Kelch (2009), Controlling the switching temperature of biodegradable, amorphous, shape-memory poly(rac-lactide)urethane networks by incorporation of different comonomers. *Biomacromolecules*, **10**(4): pp. 975–982.

Leng, J.S., H. Lv, Y. Liu and S. Du (2007), Electroactivate shape-memory polymer filled with nanocarbon particles and short carbon fibers. *Applied Physics Letters*, **91**: pp. 144105–1 to 144105–3.

Li, F.K., W. Zhu, X. Zhang, C. Zhao and M. Xu (1999), Shape memory effect of ethylene-vinyl acetate copolymers. *Journal of Applied Polymer Science*, **71**(7): pp. 1063–1070.

Li, J.Y., R.C. Rogan, E. Üstündag and K. Bhattacharya (2005), Domain switching in polycrystalline ferroelectric ceramics. *Nature Materials*, **4**(10): pp. 776–781.

Li, Y., Z.B. Hu and Y.Y. Chen (1997), Shape memory gels made by the modulated gel technology. *Journal of Applied Polymer Science*, **63**(9): pp. 1173–1178.

Liu, C.D., S.B. Chun and P.T. Mather (2002), Chemically cross-linked polycyclooctene: Synthesis, characterization, and shape memory behavior. *Macromolecules*, **35**(27): pp. 9868–9874.

Liu, C., H. Qin and P.T. (2007) Mather, Review of progress in shape-memory polymers. *Journal of Materials Chemistry*, **17**(16): pp. 1543–1558.

Liu, G.Q., X. Ding, Y. Cao, Z. Zheng and Y. Peng (2005), Novel shape-memory polymer with two transition temperatures. *Macromolecular Rapid Communications*, **26**(8): pp. 649–652.

Liu, G.Q., C.L. Guan, H. Xia, F. Guo, X. Ding and Y. Peng (2006), Novel shape-memory polymer based on hydrogen bonding. *Macromolecular Rapid Communications*, **27**(14): pp. 1100–1104.

Liu, L.L. and W. Cai (2009a), Novel copolyester for a shape-memory biodegradable material in vivo. *Materials Letters*, **63**(20): pp. 1656–1658.

Liu, Y.J., H. Lv, X. Lan, J. Leng and S. Du (2009b), Review of electro-active shape-memory polymer composite. *Composites Science and Technology*, **69**(13): pp. 2064–2068.

Loughlan, J., S.P. Thompson and H. Smith (2002), Buckling control using embedded shape memory actuators and the utilisation of smart technology in future aerospace platforms. *Composite Structures*, **58**(3): pp. 319–347.

Lu, X.L., W. Cai, Z. Gao and W.J. Tang (2007), Shape memory effects of poly(L-lactide) and its copolymer with poly(epsilon-caprolactone). *Polymer Bulletin*, **58**(2): pp. 381–391.

Lu, X.L., Z.J. Sun, W. Cai and Z.Y. Gao (2008), Study on the shape memory effects of poly(L-lactide-co-epsilon-caprolactone) biodegradable polymers. *Journal of Materials Science-Materials in Medicine*, **19**(1): pp. 395–399.

Luo, H.Y., Y. Liu, Z. Yu, S. Zhang and B. Li (2008), Novel biodegradable shape memory material based on partial inclusion complex formation between alpha-cyclodextrin and poly(epsilon-caprolactone). *Biomacromolecules*, **9**(10): pp. 2573–2577.

Luo, X.L., M. C. Zhao, M.Z. Wang, L.N. Ding and D.Z. Ma (2000), Thermally stimulated shape memory behavior of (ethylene oxide-butylene terephthalate) segmented copolymer. *Chinese Journal of Polymer Science*, **18**(4): pp. 357–361.

Maitland, D.J., M.F. Metzger, D. Schumann, A. Lee and T.S. Wilson (2002), Photothermal properties of shape memory polymer micro-actuators for treating stroke. *Lasers in Surgery and Medicine*, **30**(1): pp. 1–11.

Markland, P., Y. Zhang, G.L. Amidon and V.C. Yang (1999), A pH- and ionic strength-responsive polypeptide hydrogel: Synthesis, characterization, and preliminary protein release studies. *Journal of Biomedical Materials Research*, **47**(4): pp. 595–602.

Meng, Q.H. and J.L. Hu (2009), A review of shape memory polymer composites and blends. *Composites Part a-Applied Science and Manufacturing*, **40**(11): pp. 1661–1672.

Meng, Q.H., J. Hu, K.C. Ho, F. Ji and S. Chen (2009), The shape memory properties of biodegradable chitosan/poly(l-lactide) composites. *Journal of Polymers and the Environment*, **17**(3): pp. 212–224.

Metzger, M.F., T.S. Wilson, D. Schumann, D.L. Matthews and D.J. Maitland (2002), Mechanical properties of mechanical actuator for treating ischemic stroke. *Biomedical Microdevices*, **4**(2): pp. 89–96.

Migneco, F., Y.C. Huang, R.K. Birla and S.J. Hollister (2009), Poly(glycerol-dodecanoate), a biodegradable polyester for medical devices and tissue engineering scaffolds. *Biomaterials*, **30**(33): pp. 6479–6484.

Min, C.C., W. Cui, J. Bei and S. Wang (2005), Biodegradable shape-memory polymer-polylactideco-poly(glycolide-co-caprolactone) multiblock copolymer. *Polymers for Advanced Technologies*, **16**(8): pp. 608–615.

Mohr, R., K. Kratz, T. Weigel, M. Lucka-Gabor, M. Moneke and A. Lendlein (2006), Initiation of shape-memory effect by inductive heating of magnetic nanoparticles in thermoplastic polymers. *Proceedings of the National Academy of Sciences of the United States of America*, **103**(10): pp. 3540–3545.

Monkman, G.J. (2000), Advances in shape memory polymer actuation. *Mechatronics*, **10**(4–5): pp. 489–498.

Morshedian, J., H.A. Khonakdar, M. Mehrabzadeh and H. Eslami (2003), Preparation and properties of heat-shrinkable cross-linked low-density polyethylene. *Advances in Polymer Technology*, **22**(2): pp. 112–119.

Nagahama, K., Y. Ueda, T. Ouchi, Y. Ohya (2009), Biodegradable shape-memory polymers exhibiting sharp thermal transitions and controlled drug release. *Biomacromolecules*, **10**(7): pp. 1789–1794.

Nagata, M. and I. Kitazima (2006), Photocurable biodegradable poly(epsilon-caprolactone)/poly(ethylene glycol) multiblock copolymers showing shape-memory properties. *Colloid and Polymer Science*, **284**(4): pp. 380–386.

Nagata, M. and Y. Yamamoto (2009), Synthesis and characterization of photocross-linked poly(epsilon-caprolactone)s showing shape-memory properties. *Journal of Polymer Science Part a-Polymer Chemistry*, **47**(9): pp. 2422–2433.

Nagata, M. and Y. Yamamoto (2010), Photocurable shape-memory copolymers of epsilon-caprolactone and l-lactide. *Macromolecular Chemistry and Physics*, **211**(16): pp. 1826–1835.

Nair, D.P., N.B. Cramer, T.F. Scott, C.N. Bowman and R. Shandas (2010), Photopolymerized thiol-ene systems as shape memory polymers. *Polymer*, **51**(19): pp. 4383–4389.

Neuss, S., I. Blomenkamp, R. Stainforth, D. Blotersdorf, M. Jansen, N. Butz, A. Perez-Bouza and R. Knüchel (2009), The use of a shape-memory poly(e-caprolactone) dimethacrylate network as a tissue engineering scaffold. *Biomaterials*, **30**(9): pp. 1697–1705.

Nji, J. and G.Q. Li (2010), A biomimic shape memory polymer based self-healing particulate composite. *Polymer*, **51**(25): pp. 6021–6029.

Osada, Y. and J.P. Gong (1998), Soft and wet materials: Polymer gels. *Advanced Materials*, **10**(11): pp. 827–837.

Osada, Y. and A. Matsuda (1995), Shape-memory in hydrogels. *Nature*, **376**(6537): pp. 219–219.

Ota, S. (1981), Current status of irradiated heat-shrinkable tubing in Japan. *Radiation Physics and Chemistry*, **18**(1–2): pp. 81–87.

Peng, T., P. Gibula, K.D. Yao and M.F.A. Goosan (1996), Role of polymers in improving the results of stenting in coronary arteries. *Biomaterials*, **17**(7): pp. 685–694.

Rainer, W.C., E.M. Redding, J.J. Hitov, A.W. Sloan and W.D. Steward (1964), Heat-shrinkable polyethylene. US Patent, 3144398, Editor.

Rossi, S., F. Deflorian, A. Pegoretti, D. D'Orazio and S. Gialanella (2008), Chemical and mechanical treatments to improve the surface properties of shape memory NiTi wires. *Surface & Coatings Technology*, **202**(10): pp. 2214–2222.

Schmidt, A.M. (2006), Electromagnetic activation of shape memory polymer networks containing magnetic nanoparticles. *Macromolecular Rapid Communications*, **27**(14): pp. 1168–1172.

Schoener, C.A., C.B. Weyand, R. Murthy and M.A. Grunlan (2010), Shape memory polymers with silicon-containing segments. *Journal of Materials Chemistry*, **20**(9): pp. 1787–1793.

Schurch, K.E. and K.H.G. Ashbee (1977), Near perfect shape-memory ceramic material. *Nature*, **266**(5604): pp. 706–707.

Small, W., T. Wilson, W. Benett, J. Loge and D. Maitland (2005), Laser-activated shape memory polymer intravascular thrombectomy device. *Optics Express*, **13**(20): pp. 8204–8213.

Song, L. W. Hu, G. Wang, G. Niu, H. Zhang, H. Cao, K. Wang, H. Yang and S. Zhu (2010b), Tailored (meth)acrylate shape-memory polymer networks for ophthalmic applications. *Macromolecular Bioscience*, **10**(10): pp. 1194–1202.

Song, L., W. Hu, H. Zhang, G. Wang, H. Yang and S. Zhu (2010a), In vitro evaluation of chemically cross-linked shape-memory acrylate-methacrylate copolymer networks as ocular implants. *Journal of Physical Chemistry B*, **114**(21): pp. 7172–7178.

Takashima, K., J. Rossiter and T. Mukai (2010), McKibben artificial muscle using shape-memory polymer. *Sensors and Actuators a-Physical*, **164**(1–2): pp. 116–124.

Tamai, H., K. Igaki, E. Kyo, K. Kosuga, A. Kawashima, S. Matsui, H. Komori, T. Tsuji, S. Motohara and H. Uehata (2000), Initial and 6-month results of biodegradable poly-l-lactic acid coronary stents in humans. *Circulation*, **102**(4): pp. 399–404.

Thomsen, D.L., P. Keller, J. Naciri, R. Pink, H. Jeon, D. Shenoy and B.R. Ratna (2001), Liquid crystal elastomers with mechanical properties of a muscle. *Macromolecules*, **34**(17): pp. 5868–5875.

Vaia, R. (2005), Nanocomposites – Remote-controlled actuators. *Nature Materials*, **4**(6): pp. 429–430.

Van Humbeeck, J. (1999), Non-medical applications of shape memory alloys. *Materials Science and Engineering a-Structural Materials Properties Microstructure and Processing*, **273**: pp. 134–148.

Wada, K. and Y. Liu (2008), On the two-way shape memory behavior in NiTi alloy – An experimental analysis. *Acta Materialia*, **56**(13): pp. 3266–3277.

Wang, W.S., P. Ping, X. Chen and X. Jing (2006), Polylactide-based polyurethane and its shape-memory behavior. *European Polymer Journal*, **42**(6): pp. 1240–1249.

Wang, Y.N., H.M. Shen and M. Zhu (1991), Shape memory effect due to a phaselike transition in high-tc superconductors. *Physics Letters A*, **158**(8): pp. 413–416.

Wei, Z.G., R. Sandstrom and S. Miyazaki (1998), Shape-memory materials and hybrid composites for smart systems – Part I Shape-memory materials. *Journal of Materials Science*, **33**(15): pp. 3743–3762.

Wischke, C. and A. Lendlein (2010), Shape-memory polymers as drug carriers-a multifunctional system. *Pharmaceutical Research*, **27**(4): pp. 527–529.

Wischke, C., A.T. Neffe, S. Steuer, E. Engelhardt and A. Lendlein (2010), AB-polymer networks with cooligoester and poly(n-butyl acrylate) segments as a multifunctional matrix for controlled drug release. *Macromolecular Bioscience*, **10**(9): pp. 1063–1072.

Wischke, C., A.T. Neffe, S. Steuer and A. Lendlein (2009), Evaluation of a degradable shape-memory polymer network as matrix for controlled drug release. *Journal of Controlled Release*, **138**(3): pp. 243–250.

Wong, Y.S. and S.S. Venkatraman (2010), Recovery as a measure of oriented crystalline structure in poly(L-lactide) used as shape memory polymer. *Acta Materialia*, **58**(1): pp. 49–58.

Wong, Y.S., Y. Xiong, S.S. Venkatraman and F.Y. Boey (2008), Shape memory in uncross-linked biodegradable polymers. *Journal of Biomaterials Science-Polymer Edition*, **19**(2): pp. 175–191.

Xie, T. (2010), Tunable polymer multi-shape memory effect. *Nature*, **464**(7286): pp. 267–270.

Xue, L.A., S.Y. Dai and Z. Li (2010), Biodegradable shape-memory block co-polymers for fast self-expandable stents. *Biomaterials*, **31**(32): pp. 8132–8140.

Yang, B., W.M. Huang, C. Li, C.M. Lee and L. Li (2004), On the effects of moisture in a polyurethane shape memory polymer. *Smart Materials & Structures*, **13**(1): pp. 191–195.

Yu, X.J., S. Zhou, X. Zheng, Y. Xiao and T. Guo (2009), Influence of in vitro degradation of a biodegradable nanocomposite on its shape memory effect. *Journal of Physical Chemistry C*, **113**(41): pp. 17630–17635.

Yu, Y.L. and T. Ikeda (2005), Photodeformable polymers: A new kind of promising smart material for micro- and nano-applications. *Macromolecular Chemistry and Physics*, **206**(17): pp. 1705–1708.

Zeng, M., S.W. Or and H.L.W. Chan (2010), Magnetic field-induced strain and magnetoelectric effects in sandwich composite of ferromagnetic shape memory Ni-Mn-Ga crystal and piezoelectric PVDF polymer. *IEEE Transactions on Ultrasonics Ferroelectrics and Frequency Control*, **57**(10): pp. 2147–2153.

Zhang, H., H. Wang, W. Zhong and Q. Du (2009a), A novel type of shape memory polymer blend and the shape memory mechanism. *Polymer*, **50**(6): pp. 1596–1601.

Zhang, S.F., Y. Feng, L. Zhang, J. Sun, X. Xu and Y. Xu (2007), Novel interpenetrating networks with shape-memory properties. *Journal of Polymer Science Part a-Polymer Chemistry*, **45**(5): pp. 768–775.

Zheng, X.T., S. Zhou., X. Yu, X. Li, B. Feng, S. Qu and J. Weng (2008), Effect of in vitro degradation of poly(D,L-lactide)/beta-tricalcium composite on its shape-memory properties. *Journal of Biomedical Materials Research Part B-Applied Biomaterials*, **86B**(1): pp. 170–180.

Zheng, X.T., S. Zhou, Y. Xiao, X. Yu, X. Li and P. Wu (2009), Shape memory effect of poly(D,L-lactide)/Fe_3O_4 nanocomposites by inductive heating of magnetite particles. *Colloids and Surfaces B-Biointerfaces*, **71**(1): pp. 67–72.

Zheng, X.T., S.B. Zhou, X. Li and J. Weng (2006), Shape memory properties of poly(D,L-lactide)/hydroxyapatite composites. *Biomaterials*, **27**(24): pp. 4288–4295.

Zhou, S.B., X. Zheng, X. Yu, J. Wang, J. Weng, X. Li, B. Feng and M. Yin (2007), Hydrogen bonding interaction of poly(D,L-lactide)/hydroxyapatite nanocomposites. *Chemistry of Materials*, **19**(2): pp. 247–253.

Zhu, G., G. Liang, Q. Xu and Q. Yu (2003), Shape-memory effects of radiation cross-linked poly(epsilon-caprolactone). *Journal of Applied Polymer Science*, **90**(6): pp. 1589–1595.

Zhu, Y., J.L. Hu, K.W. Yeung, Y.Q. Liu and H.M. Liem (2006), Influence of ionic groups on the crystallization and melting behavior of segmented polyurethane ionomers. *Journal of Applied Polymer Science*, **100**(6): pp. 4603–4613.

Zhu, Y., J. Hu, K.-F. Choi, Q. Meng, S. Chen and K.-W. Yeung (2008), Shape memory effect and reversible phase crystallization process in SMPU ionomer. *Polymers for Advanced Technologies*, **19**(4): pp. 328–333.

Zini, E., M. Scandola, P. Dobrzynski, J. Kasperczyk and M. Bero (2007), Shape memory behavior of novel (L-Lactide- glycol ide-trimethylene carbonate) terpolymers. *Biomacromolecules*, **8**(11): pp. 3661–3667.

8

Smart polymer hydrogels: properties, synthesis and applications

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Abstract: Smart hydrogel systems with various chemically and structurally responsive moieties exhibit responsiveness to external stimuli including temperature, pH, ionic concentration, light, magnetic fields, electrical fields and chemicals. Polymers with multiple responsive properties have also been developed elegantly combining two or more stimuli-responsive mechanisms. Smart polymer hydrogels change their structural and volume phase transition as a response to external stimuli resulting in an enormous potential for scientific observations and for various advanced technological applications. This chapter will emphasize the most recent advances in the field. Further, developments of different smart hydrogels including their preparation and biomedical applications will be discussed in depth.

Key words: smart hydrogels, stimuli-responsive, polymers, drug delivery, tissue engineering.

8.1 Introduction

Hydrogels are polymeric networks exhibiting a high level of hydration and three-dimensional (3D) microstructures showing resemblance to natural tissue (Cushing and Anseth, 2007; Kopecek, 2002; Kumar *et al*., 2007). 'Smart' hydrogels can respond reversibly to external stimuli including pH, temperature, electrical fields, light, pressure, ionic strength, solvent, etc. They were among the first biomaterials designed for clinical use in the early 1960s by Otto Wichterle and Drahoslav Lím (Kopecek, 2009). During the past two decades, they have attracted a great deal of interest due to their potential applications in various fields.

Interestingly, the incorporation of some stimuli-responsive co-monomers, either into the backbone of the network or as pendant groups, leads to the preparation of smart hydrogels that can be responsive to various stimuli. A dynamic response is crucial for applications involving smart hydrogels since conventional hydrogels show low response rates in general. Hydrogels

possessing such 'sensing' properties can undergo reversible volume phase transitions or sol–gel phase transitions upon changes in the environmental conditions. The stimuli-responsive properties result in a large number of possible applications including controlled drug release, tissue engineering, soft machines, etc. (Bajpai *et al*., 2008; Ilg, 2013). Among these applications, these 'intelligent' or smart polymers have made remarkable progress in drug delivery applications since they have played an important role in controlling not only where a drug is delivered, but also when and at what interval it is released.

The stimuli to which smart hydrogels respond are commonly classified in three categories: physical, chemical or biological (Ahn *et al*., 2008; Peppas *et al*., 2006). Physical stimuli indicate response towards light, temperature, ultrasound, magnetic, mechanical, or electrical fields; chemical stimuli indicate solvent, ionic strength, electrochemical fields, pH; and biological stimuli relate to the actual functioning of molecules such as enzymatic reactions and receptor recognition of molecules. As indicated above, hydrogels can also be designed to respond to multiple stimuli, that is, simultaneously respond to more than one stimulus.

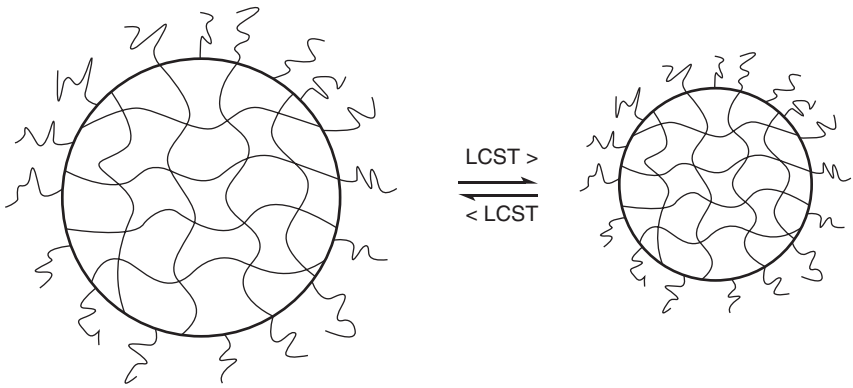
8.2 Key types and properties of smart polymer hydrogels

Several types of smart hydrogels exist including those which react to temperature, pH, light, electricity, ions, glucose or are multi-responsive materials. In this section, an overview will be given of the different types of smart hydrogels together with their specific properties and some recent, state-of-the-art examples for each hydrogel class.

8.2.1 Temperature-responsive hydrogels

Temperature-responsive smart hydrogels change their structural properties in response to the temperature of their environment (Fig. 8.1). They comprise the most commonly studied responsive systems having great potential for various biomedical applications. Temperature-responsive hydrogels can be classified as negatively thermosensitive lower critical solution temperature (LCST) and positively thermosensitive upper critical solution temperature (UCST) polymers (Ishida *et al*., 2012; Qiu and Park, 2001).

Negatively, temperature-sensitive hydrogels possess a LCST and contract upon heating above the LCST. This type of swelling behavior is known as the inverse (or negative) temperature dependence. Inverse temperature-dependent hydrogels are comprised of polymer chains which either possess moderately hydrophobic groups or a mixture of hydrophilic



8.1 Representation of temperature-responsive smart hydrogels showing LCST behavior.

and hydrophobic segments. In case too hydrophobic polymer chains would be present, no dissolution in water whatsoever could occur. At lower temperatures, hydrogen bond formation between hydrophilic segments in the polymer chain and water molecules dominates, leading to enhanced dissolution. With increasing temperature, interactions among hydrophobic segments gain in importance, while hydrogen bond formation is reduced. As a result, the hydrogel shrinks due to the interpolymer chain associations corresponding to the hydrophobic interactions. Interestingly, with increasing amounts of hydrophobic constituents present in the polymer backbone, the LCST is decreased (Huber *et al*., 2008). In general, LCST systems are mainly relevant when aiming at controlled drug release and, in particular, for the release of proteins (Bromberg and Ron, 1998; Hassouneh *et al*., 2012). Copolymers constituting (N-isopropylacrylamide) (PNIPAAm) are usually applied as LCST polymers. PNIPAAm-based hydrogels show an on/off drug release at low and high temperatures, respectively, enabling pulsatile drug release (Prabaharan and Mano, 2006; Satarkar *et al*., 2010; Trongsatitkul and Budhlall, 2013).

A positively temperature-sensitive hydrogel is characterized by an UCST and these hydrogels contract upon cooling below the UCST. Polymer networks consisting of poly(acrylic acid) (PAA) and polyacrylamide (PAAm) show such positive temperature-dependent swelling (Ward and Georgiou, 2011).

The most commonly used thermo-responsive materials include those prepared starting from poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) (Pluronics®, Tetronics®, poloxamer) (Bromberg, 2001; Fernandez-Tarrio *et al*., 2008). Depending on the Pluronic type, the polymer solution is a free-flowing liquid at ambient temperature and a gel at body

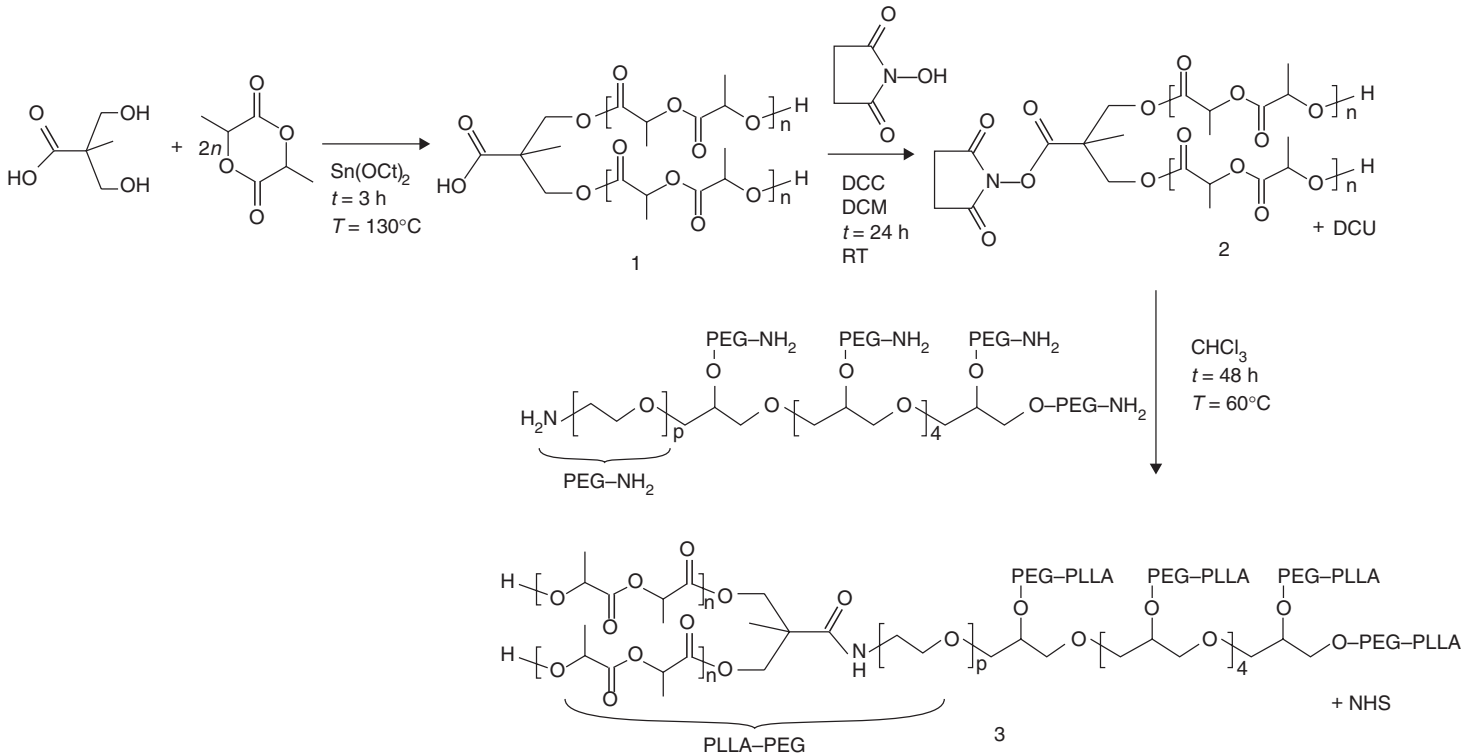
temperature. Temperature-responsiveness can thus be achieved by incorporating or grafting temperature-responsive moieties including Pluronic F127 or PNIPAAm. Temperature-sensitive hydrogels can also be developed using temperature-sensitive cross-linking agents. For example, a hybrid hydrogel system has already been assembled from water soluble synthetic polymers and a well-defined protein-folding motif (i.e., the coiled coil) (Hamcerencu *et al*., 2009 ; Wang *et al*., 1999). Interestingly, the hydrogel thus developed underwent temperature-induced collapse due to the cooperative conformational transition. Temperature-sensitive cross-linking agents can thus add a new dimension when designing temperature-sensitive hydrogels.

Ishida *et al.* (2012) synthesized temperature-responsive PNIPAAm hydrogels with movable cross-linking points via the radical copolymerization of with cyclic poly(ethylene glycol) (PEG). The resulting hydrogel exhibited fast volume shrinking due to the increased mobility of the polymer chains. Velthoen *et al.* (2011) synthesized a highly branched PEG-b- (L-lactide) block copolymer using trifunctional PLLA's and amine functionalized PEG (Fig. 8.2). The copolymers obtained showed thermo-responsive gelation behavior at polymer solution concentration of ≥ 4 wt%. Interestingly, the transition temperature could be fine-tuned by changing the copolymer concentration and the molecular weight of the poly(L-lactide) blocks applied. The hydrogels developed served as injectable systems enabling *in situ* gel formation.

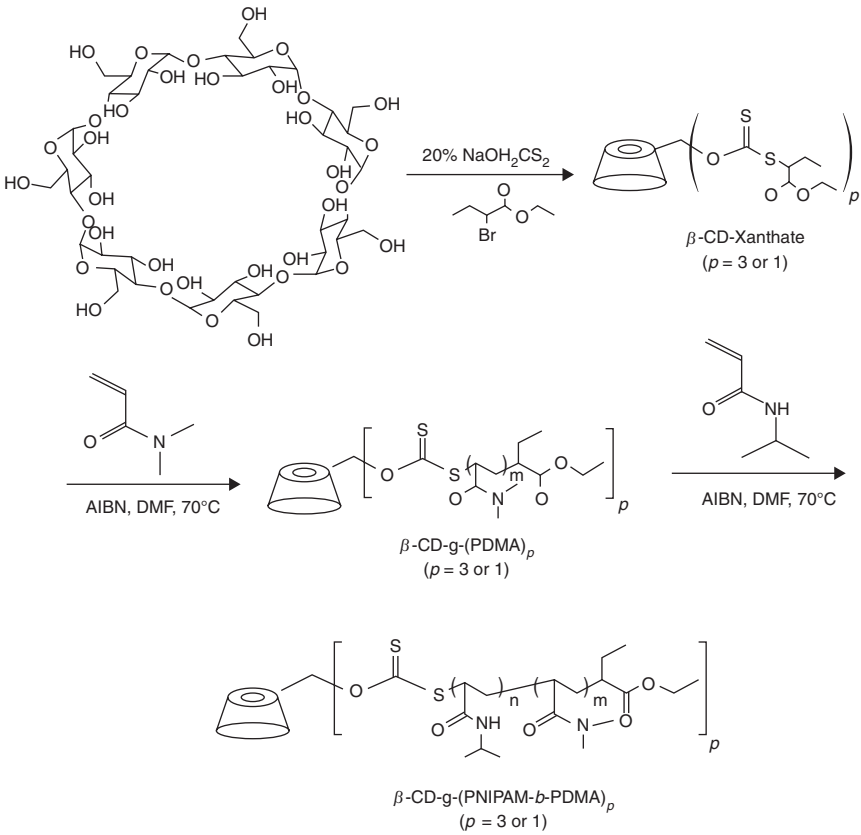
Zhang *et al*. (2012) synthesized a temperature-responsive hydrogel system composed of a three-arm star copolymer using a β-cyclodextrin (β-CD) core. β-CD xanthate was used as a chain transfer agent and the star-shaped copolymer was synthesized using a sequential reversible addition-fragmentation polymerization (RAFT) method (Fig. 8.3). The arms of this star-shaped copolymer consisted of hydrophilic poly(*N*,*N*-dimethylacrylamide) (PDMA) blocks and temperature-responsive PNIPAAm blocks. Below the LCST of the PNIPAM segment, both blocks are water soluble thus the copolymer is soluble. However, above the LCST, the PNIPAM blocks become water-insoluble and therefore aggregate. The specific star-shaped topology and the thermally collapsed PNIPAAm chains were responsible for the gelation behavior which imparted exciting properties to the hydrogel developed.

8.2.2 pH-responsive smart hydrogels

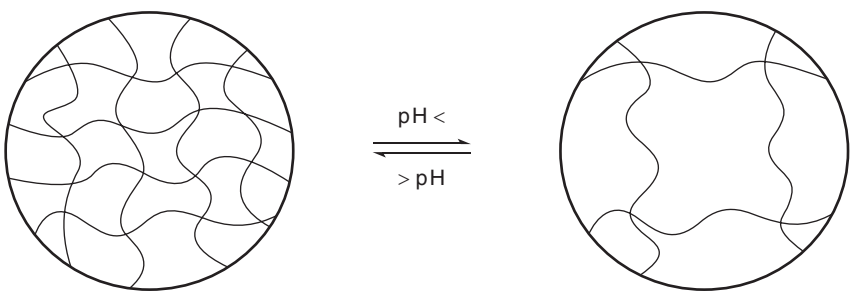
pH-responsive hydrogels are synthesized from pH-sensitive polymers possessing ionizable functional groups which either accept or release protons in response to changes in environmental pH (Fig. 8.4) (Dou *et al*., 2012; Krogsgaard *et al*., 2013; Schoener *et al*., 2012). The structural properties of these hydrogel types are dramatically altered above and below a predetermined pH. The rapid change in the net charge of pendant or backbone



8.2 Synthesis scheme of poly(L-lactide) (PLLA) and 8PEG–PLLA copolymer. DCC – dicyclohexylcarbodiimide; DCU – dicyclohexylurea.



8.3 A representative reaction route for the synthesis of β-CD-g-(PNIPAM-b- PDMA) and β-CD-g-(PNIPAM-b-PDMA)3 via RAFT using β-CD as the macromolecular chain transfer agent.



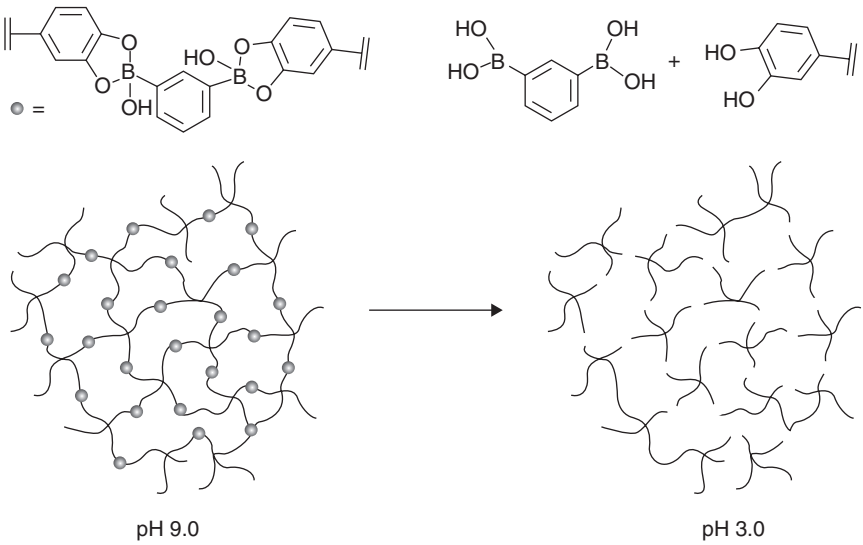
8.4 Representation of a pH-responsive smart hydrogel.

functionalities with respect to pH causes an alternation of the hydrodynamic volume or the conformation of the polymer chains.

Most anionic pH-sensitive polymers are based on PAA (Carbopol®, carbomer) or derivatives thereof including poly(methacrylic acid) (PMAA) and poly(diethylaminoethyl methacrylate) (PDEAEMA) (Soppimath *et al*., 2002). In addition, some polymers containing phosphoric acid derivatives have also been reported (Miyata *et al*., 1994; Nakamae *et al*., 1992). These polymers, comprising a large number of ionizable functional groups, are generally referred to as polyelectrolytes. The presence of ionizable groups onto polymer chains results in increased hydrogel swelling compared to non-electrolyte polymer hydrogels. Since swelling of polyelectrolyte hydrogels is mainly due to the electrostatic repulsion occurring among charges present on the polymer backbone, the swelling extent is influenced by factors reducing electrostatic repulsions such as pH, ionic strength and the type of counter ions present. The pH-responsive nature of hydrogels can be applied for biomolecule delivery in neutral or alkaline environments. Polymers such as chitosan, poly(ethylene imine) (PEI), poly(dimethylamino-ethyl-methacrylate) (PDMAEMA) and PAA possess basic functional moieties including primary, secondary and tertiary amines that become ionized as the pH decreases. Jain *et al.* (2007) investigated the pH-responsiveness of PAA after the incorporation of acetal or ketal linkages into the backbone. As anticipated, acetal and ketal linkages resulted in the degradation of the polymer into low molecular weight hydrophilic compounds upon lowering the pH. The developed polymers demonstrated a pH-dependent degradation profile with a significant increase in hydrolysis rate as the pH was lowered from 7.4 to 5.0, the pH commonly found in lysosomes.

He *et al*. (2011) synthesized a pH-responsive hydrogel based on boronate–catechol complexation using 1,3-benzenediboronic acid and catechol end groups present on 4-arm PEG catechol (cPEG) (Fig. 8.5). Under basic aqueous conditions, the 1,3-benzenediboronic acid establishes a tetrahedral borate ester with the catechol end moieties of 4-arm PEG catechol and forms a three-dimensional, pH-responsive smart hydrogel.

Wang *et al.* (2012) developed a pH-responsive smart hydrogel using poly(lactic acid) (PLA), methoxyl poly(ethylene glycol) (MPEG) and itaconic acid (IA) (P(LE-IA-MPEG)) via heat-initiated free-radical polymerization in the absence of organic solvents (Fig. 8.6). The effect of the pH value on the swelling ratio was determined in buffers with pH ranging from 1.2 to 6.8. At low pH (i.e., 1.2), many hydrogen bonds exist due to the presence of non-dissociated carboxylic acids in the hydrogel. As a result, these hydrogen bond complexes restrict the movement or relaxation of the network chains present. Upon raising the pH to 6.8, the carboxylic acid moieties become partially ionized, which results in the destruction of hydrogen



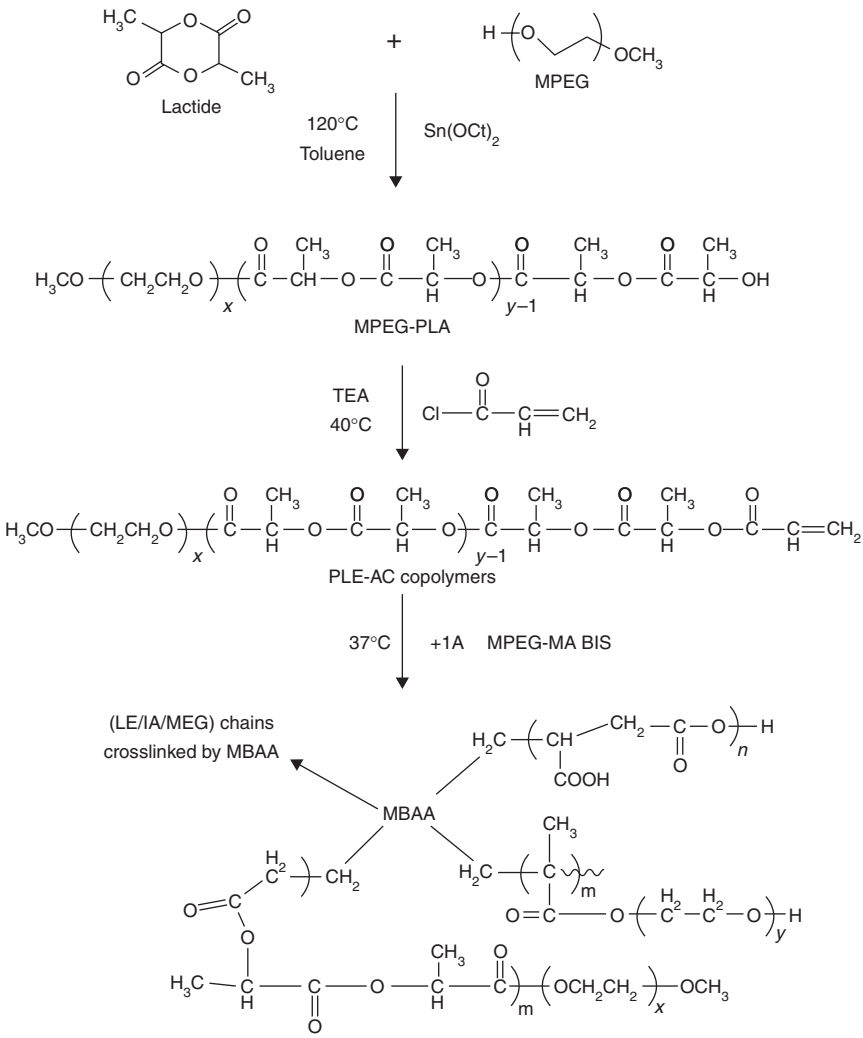
8.5 Schematic illustration of a pH-responsive hydrogel based on cPEG and 1,3-benzenediboronic acid in aqueous solution at 20°C.

bonds and the creation of electrostatic repulsions among the polymer chains, leading to hydrogel swelling.

Yoshikawa *et al.* (2011) synthesized a triblock copolymer, pH-responsive smart hydrogel consisting of pH-sensitive poly(2-(diisopropylamino)ethyl methacrylate) (PDPA) and biocompatible poly(2-(methacryloyloxy)-ethyl phosphorylcholine) (PMPC) (Fig. 8.7). These hydrogels allowed fine-tuning of the mechanical environment experienced by mouse myoblast cells. The hydrogel elasticity could be regulated via precise pH adjustment without adversely affecting cell viability. The myoblast cells exhibited pronounced stress fiber formation and flattening upon increasing the hydrogel elasticity. Interestingly, this concept can be utilized to monitor how cells adapt their morphology with respect to changes in their mechanical environment.

8.2.3 Light-responsive hydrogels

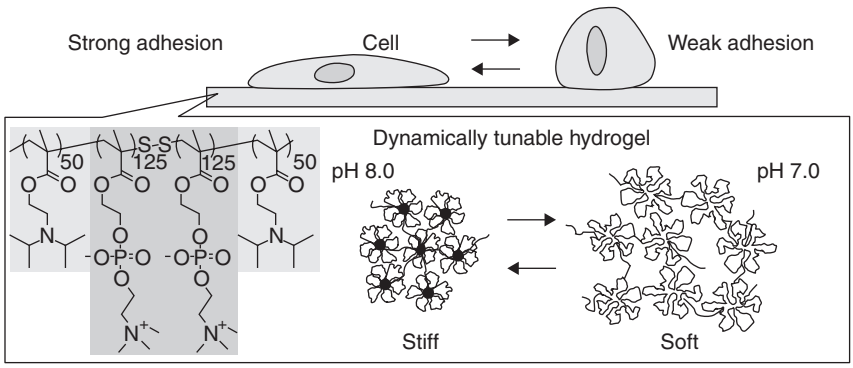
Light-responsive smart hydrogels are composed of a polymeric network possessing light reactive groups such as photochromic moieties. Upon light irradiation, these hydrogels change their physical and/or chemical properties including elasticity, viscosity, shape and swelling degree. Several approaches have already been developed to incorporate photochromic moieties into hydrogels including physically (i.e., non-covalently) cross-linked, chemically



8.6 The synthesis of a P(LE-IA-MPEG) hydrogel.

(i.e., covalently) cross-linked and many others (Alvarez-Lorenzo *et al.*, 2009; Yan *et al.*, 2012; Zhao and Stoddart, 2009).

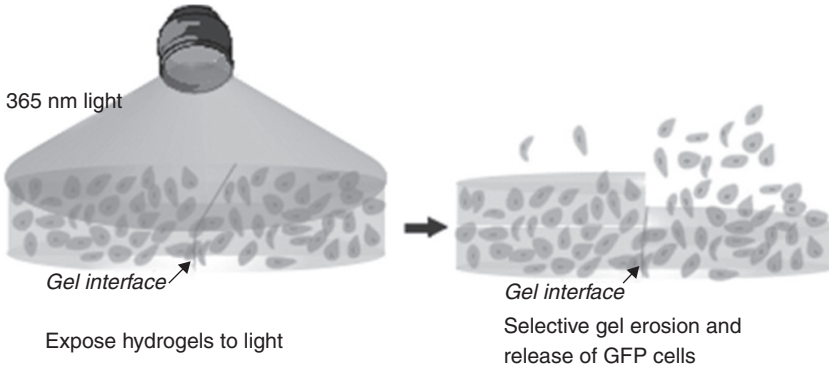
Light-sensitive hydrogels show potential applications when developing optical switches, display units and ophthalmic drug delivery devices. The light stimulus can be imposed instantly and delivered in specific doses with high accuracy, rendering these light-sensitive hydrogels advantageous over others. The capacity for instantaneous delivery upon applying a stimulus makes the development of light-sensitive hydrogels important for various applications



8.7 Scheme of pH-sensitive PDPA50-PMPC250-PDPA50 triblock copolymer hydrogel.

in both engineering as well as biochemical fields. Light-sensitive hydrogels can be classified into UV-sensitive and visible light-sensitive hydrogels. Zhu *et al*. (2012) demonstrated a facile and straightforward method for the preparation of PNIPAM/graphene oxide (GO) nanocomposite hydrogels by *in situ* γ-irradiation-assisted polymerization of an aqueous solution of *N*-isopropylacrylamide and GO. The combination of GO with PNIPAM resulted in excellent photothermal properties, where the reversible phase transition of the hydrogel was remotely controlled by laser exposure or non-exposure. The light-activated nanocomposite hydrogels, showing excellent photothermal sensitivity, could extend potential application not only in the biomaterials field, but also for microdevices. For example, a composite polypeptide (PC10P) hydrogel with gold nanorods exhibited unique features suitable for on-demand changes in gels. These gels underwent instantaneous thermal transitions, by application of external near infra-red (NIR) light, providing a technique to regulate drug release.

Recently, Mabrouk *et al*. (2009) reported on a light-responsive system consisting of polymeric vesicles in the micrometer size range. The vesicles were composed of poly(ethylene glycol)-b-polybutadiene (PEG-b-PBD) and a liquid crystal based copolymer, poly(ethylene glycol)-b-poly(4-butyloxy-2′-(4-(methacryloyloxy)butyloxy)-4′-(4-butyloxybenzoyloxy)azobenzene) (PAzo). The PEG–PBD copolymers are segregated in the inner leaflet of the membrane, while the PAzo copolymers compose the outer leaflet of the membrane. When the azo moieties are in the *trans* form, the PAzo polymer adopts a rod-like structure in the membrane. When light is switched on, the azo moieties are in the *cis* form, and the PAzo polymers undergo a conformational change to reach a coil conformation. As a result, the volume occupied by the PAzo chains increases, leading to a spontaneous change in curvature and to bursting of the giant vesicles by 'curling' of the membrane.



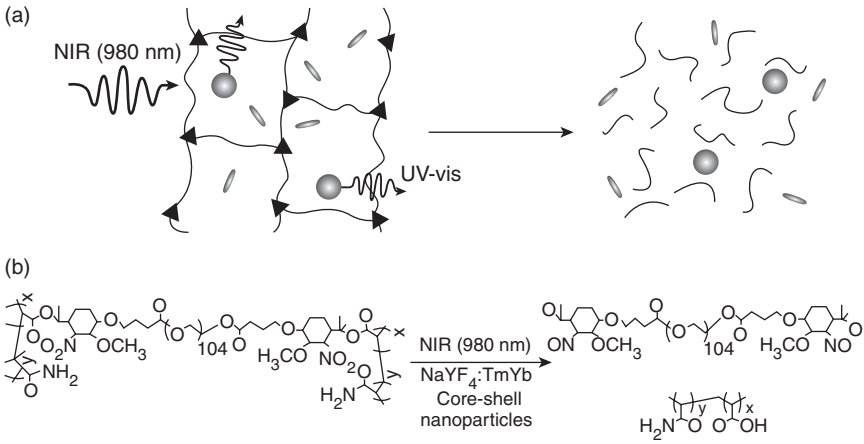
8.8 Photosensitive release of encapsulated RET fused gene (RFG-) or GFP-expressing hMSCs.

Griffin and co-workers synthesized photoresponsive smart hydrogels containing photodegradable ortho-nitrobenzyl (o-NB) groups in the macromer backbone via redox polymerization. The apparent rate constants of the degradation were quantified using photorheology (at 370 nm, 10 mW/cm^2). Upon decreasing the number of aryl ethers on the o-NB group or changing the functionality from primary to secondary at the benzylic site, the degradation constant increased dramatically. The results also demonstrate that the hydrogels can be used to encapsulate and release human mesenchymal stem cells (hMSCs) without compromising cell viability (Fig. 8.8) (Griffin and Kasko, 2012).

Yan *et al*. (2012) synthesized a photoresponsive hybrid up-conversion nanoparticles (UCNPs) hydrogel system that represents the first demonstration of applying the multiphoton effect of UCNPs to trigger structural changes in photosensitive hydrogels. It enables the application of continuous-wave NIR light (980 nm) to induce the gel–sol transition and release large, biomacromolecules such as proteins and enzymes entrapped within the hydrogel into an aqueous solution 'on demand', while preserving their bioactivity (Fig. 8.9). This study leads to a new development in harnessing the unique properties of UCNPs for photosensitive hydrogels which are of biological and biomedical interest.

8.2.4 Electro-responsive smart hydrogels

Electrically responsive smart hydrogels are capable of executing mechanical work including expansion, contraction, elongation and bending under the influence of an electric field depending on the hydrogel shape and its position relative to the electrodes (Bünsow and Johannsmann, 2008; Kulkarni



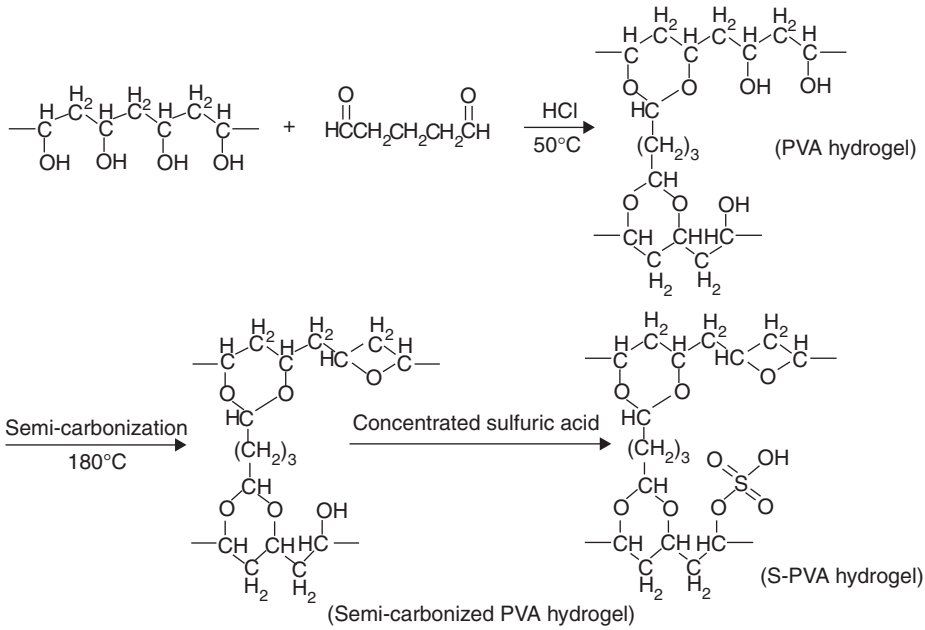
8.9 (a) Schematic illustration of the NIR light-triggered degradation of a photosensitive hydrogel using the UV light generated by encapsulated UCNPs. The polymeric components are depicted as black lines, the photocleavable cross-links as triangles, the UCNPs as spheres and the trapped biomacromolecules as rods. (b) Chemical structure of the hydrogel containing photocleavable o-nitrobenzyl moieties in the cross-linker and the NIR light-induced photoreaction of the hydrogel via UV light emitted by loaded NaYF4:TmYb core–shell UCNPs. The number of monomer units per PEG cross-linker was 104, and the molar ratio between the acrylamide monomer units and the PEG cross-linker, y/x, was ~50 (as determined from the ^{1}H NMR spectrum of a fully UV-degraded gel sample in deuterated chloroform(CDCl3)).

and Sa, 2009; Murdan, 2003). De-swelling or contraction is observed when a hydrogel lies perpendicular to the electrodes, or the gels are in contact with the electrodes. Bending can be observed when the gel is positioned in parallel without contacting the electrodes. If the hydrogel surface is in contact with the electrode, the result of applying an electric field to the hydrogel may be different from systems for which the hydrogel is placed in water (or in other solvents) without touching the electrode. Hydrogel bending has been widely investigated for the production of mechanical devices including artificial muscles, valves, switches, 'soft actuators' and 'molecular machines' (Messing and Schmidt, 2011). De-swelling or contractile behavior of hydrogels has been mainly studied for controlled drug delivery. An electric field as the external stimulus offers certain advantages such as precise control with regard to the current magnitude, the duration of the electric pulses, the interval between pulses, etc. Some considerable time ago, evidence was observed on the use of electric currents *in vivo* in the form of ionotophoresis and electroporation in the field of dermal and transdermal drug delivery,

and the safe limits of electric field strengths for topical applications were determined (Denet *et al*., 2004).

Electrically responsive smart hydrogels are prepared from polymers which contain relatively high concentrations of ionisable groups along the backbone and are thus both pH-responsive as well as electro-responsive. Both synthetic as well as naturally occurring polymers, either separately or in combination, have already been explored. Examples of naturally occurring polymers include hyaluronic acid, chondroitin sulfate and agarose. Synthetic polymers applied are mostly (meth)acrylate based. In general, electrically responsive polymers are conducting polymers. For example, polythiophene or sulfonated-polystyrene shows swelling, shrinking or bending in response to an externally applied field (Kumar *et al*., 2007). Among many electro-responsive polymers, electro-responsive hydrogels have become appealing both because of their use for controlled drug delivery as well as their biocompatibility. In 1982, Tanaka and his group observed an electro-responsive contraction and phase separation of a partially hydrolyzed polyacrylamide gel in contact with platinum electrodes. They observed de-swelling due to an electrophoretic pressure gradient (Tanaka *et al*., 1982). A similar effect for water-swollen poly(2-acrylamido-2-methyl-1-propanesulfonic acid) gel was observed by Osada and Hasebe in 1985. They noticed up to 30% loss of absorbed water from a gel in the presence of an electric field (Osada and Hasebe, 1985).

Ramanathan and Block (2001) evaluated and characterized the use of chitosan gels as matrices for electrically modulated drug delivery. In electrification studies, release-time profiles for neutral (hydrocortisone), anionic (benzoic acid) and cationic (lidocaine hydrochloride) drugs from hydrated chitosan gels were monitored in response to different currents as a function of time. Using a similar approach, chondroitin-4-sulfate hydrogels were examined by Jensen *et al*. (2002) as potential matrices enabling electro-controlled peptide and protein delivery. Rahimi *et al*. (2012) developed a 3D semi-interpenetrating network composed of PAA and fibrin electro-responsive smart hydrogels by the incorporation of biodegradable fibrin into a network of electro-sensitive anionic PAA developed through free-radical polymerization and subsequent cross-linking using ammonium persulfate (APS), tetramethylethylenediamine (TMEDA), and N,N-methylenebisacrylamide (MBAA) as initiator, accelerator and cross-linking agent, respectively. The electrical hydrogel stimulation resulted in an improved cell penetration and alignment within the tissue construct. This system could be used to improve culture and seeding conditions for manufacturing tissue-engineered vascular grafts, which can both enhance cell migration as well as facilitate perfusion of cell culture medium throughout the scaffold by applying a regular stimulation pattern (Rahimi *et al*., 2012).

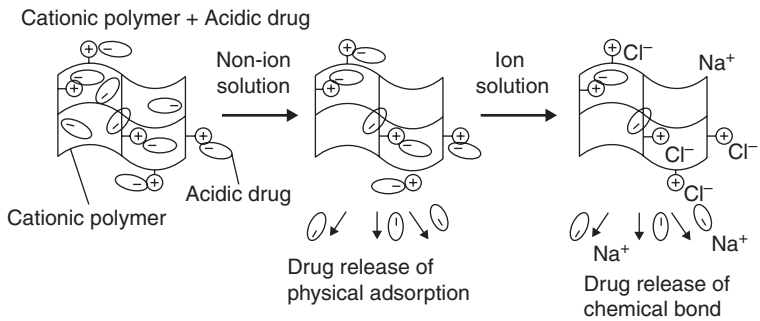


8.10 Chemical reaction flow chart for the preparation of the S-PVA hydrogel.

Yang *et al.* (2010) designed a novel sulfonated poly(vinyl alcohol) (S-PVA) electro-responsive smart hydrogel by the sulfonation of a semicarbonized poly(vinyl alcohol) using concentrated sulfuric acid (Fig. 8.10). In the presence of a non-contact direct current (DC) electric field, the hydrogel strip showed electro-responsive behavior. The bending behavior could be adjusted via the voltage of the applied electric field and the ionic strength of the electrolyte, and resulted in potential applications as electrodriven chemomechanics, artificial muscles and actuators. With repeated changes in the direction of the applied potential, the hydrogel strip exhibited a reversible bending behavior.

8.2.5 Ionic-responsive cationic polymers

In many biological processes, ions play a crucial role. Thus, the utilization of ion-sensitive polymeric hydrogels could dramatically improve their therapeutic potential (Chen *et al.*, 1997; Rasool *et al.*, 2010; Zhao and Moore, 2001). Ionic-responsiveness of polymers refers to their property of undergoing relatively large and abrupt physical or chemical changes in response to small external changes in the ion concentration. The ionic concentration of solvents holds the key role in the interactions between polymeric chains



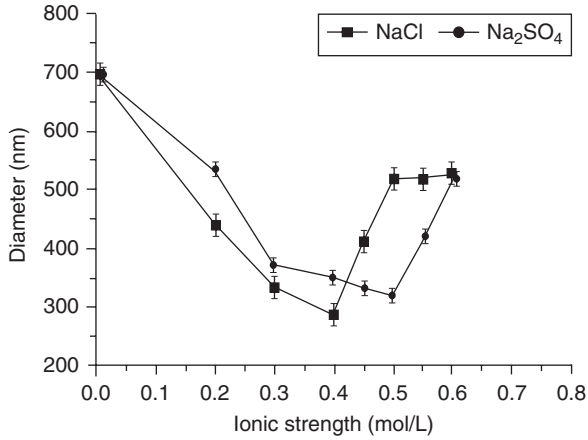
8.11 The mechanism of ion-mediated drug release from ionic polymers at different ionic strengths. (*Source*: Adapted with permission from Sutani *et al.* (2002). © 2002 Elsevier Science Limited.)

and solvents and the final molecular conformation. Ion-sensitive properties have been shown, among others, for a P(DMAEMA-co-acrylic acid) copolymer which forms a stable ionic complex with methylene blue and exhibits ion-sensitive drug release properties. Upon exposing this copolymer hydrogel to an isotonic sodium chloride solution, the drug was released from the polymer and exhibited a constant drug release profile different from the release in an aqueous medium (Fig. 8.11) (Sutani *et al.*, 2002).

The cationic amine functionalities of PDMAEMA are partly protonated in aqueous solution and the electrostatic repulsion among the repeating units of PDMAEMA results in a more expanded conformation due to the enhanced chain mobility. With the increase in ionic strength by the addition of NaCl, less repulsion occurs and a more coiled conformation is anticipated. PDMAEMA-g-PEG hydrogel nanoparticles have also been shown to have ion-sensitive properties. The size of the cationic nanoparticles decreased with increasing ionic strength due to the decrease of the osmotic pressure within the polymeric networks. Further increase of the ionic strength resulted in the destruction of the hydrogen bonds present and resulted in hydrogel nanoparticle aggregation and thus a size increase (Fig. 8.12) (Deng *et al.*, 2008). In a recent study, Liu *et al.* (2013) developed P(NIPAM-co-CE) ion-sensitive smart hydrogels wherein the crown ether units present contributed to the ionic response (Fig. 8.13).

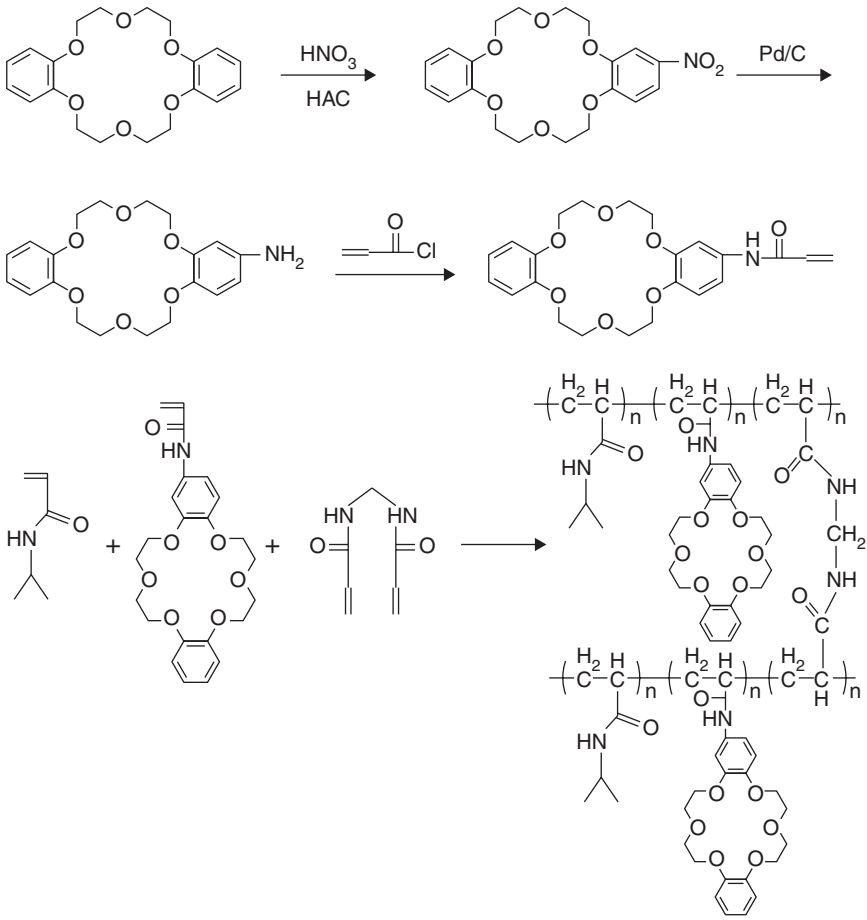
8.2.6 Glucose-sensitive hydrogels

Intelligent stimuli-responsive delivery systems using hydrogels that can release insulin are referred to as glucose-sensitive hydrogels and are a field of intensive research (Ehrick *et al.*, 2009; Kim and Park, 2001; Traitel *et al.*, 2000). One of the challenges in the controlled drug delivery area includes the



8.12 Effect of ionic strength on swelling behavior of cationic (PDMAEMA-g-PEG) nanoparticles. (*Source*: Adapted with permission from Deng *et al*. (2008). © 2008 Elsevier Limited.)

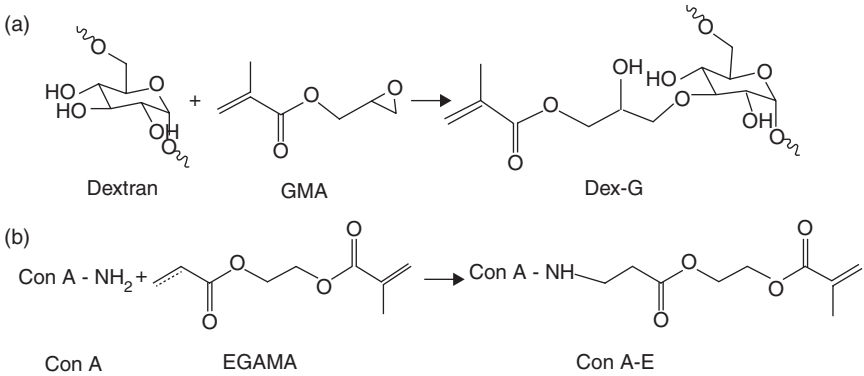
development of self-regulated and/or modulated insulin delivery systems. Insulin has to be delivered in an exact amount at the exact time of need, which requires a self-regulated insulin delivery system possessing glucose-sensing ability and an automatic shut-off mechanism. Many hydrogel systems which have a glucose sensor built into the system have already been developed for modulating insulin delivery. pH-sensitive polymers containing immobilized insulin and glucose oxidase can swell in response to a specific blood glucose level enabling insulin release in a pulsatile fashion. Another approach is based on the competitive binding of insulin or insulin and glucose to a fixed number of binding sites in concanavalin A (Con A), where insulin is displaced in response to glucose stimuli, thus functioning as a self-regulating insulin delivery system. Con A has also been frequently used in modulated insulin delivery. Con A is a glucose-binding protein obtained from the jack bean plant, *Canavalia ensiformis*. In this type of system, insulin molecules are attached to a support or carrier through specific interactions which can be interrupted by glucose itself. Zhang *et al*. (2006) prepared D-glucose-sensitive hydrogel membranes based on cross-linking carboxymethyl dextran with the glucose-binding lectin Con A using carbodiimide chemistry. Protein diffusion studies indicated that the hydrogel permeability increased in response to changes in the D-glucose concentration of the external medium, causing competitive displacement of the affinity cross-links. Glucose-selective optical sensors were fabricated by incorporating 3-phenylboronic acid and a tertiary amine, dimethylaminopropylacrylamide, into a hydrogel matrix. Determination of glucose in solution is based on the glucose-induced hydrogel contraction. In that work, the gel was fabricated at the end of an optical fiber, and an



8.13 The synthetic scheme of p(NIPAM-co-CE) hydrogels.

interferometric technique was used to measure the optical length. The results also showed that there was negligible interference for glucose measurements at normal blood-lactate levels of 1 mM; however, interference increased when the lactate concentration increased above 5 mM. The sensor showed a very good response to glucose variations in *ex vivo* blood plasma that was collected in the presence of ethylene diamine tetraacetic acid (EDTA) as anticoagulant.

Zhang *et al*. (2008) developed a new comb-type, glucose-responsive poly(NIPAM-co-AAPBA) grafted hydrogel with rapid response to changes in blood glucose concentration at physiological temperature. This polymeric hydrogel contained thermo-responsive poly(*N*-isopropylacrylamide) (PNIPAM) groups as actuators and phenyl boronic acid (PBA) as glucose-

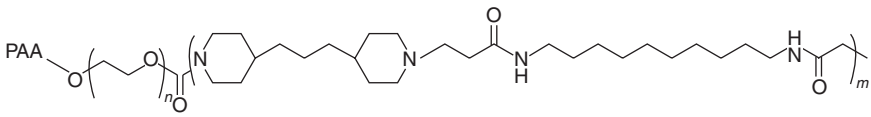


8.14 Reaction scheme for the preparation of (a) Dex-G and (b) Con A–E.

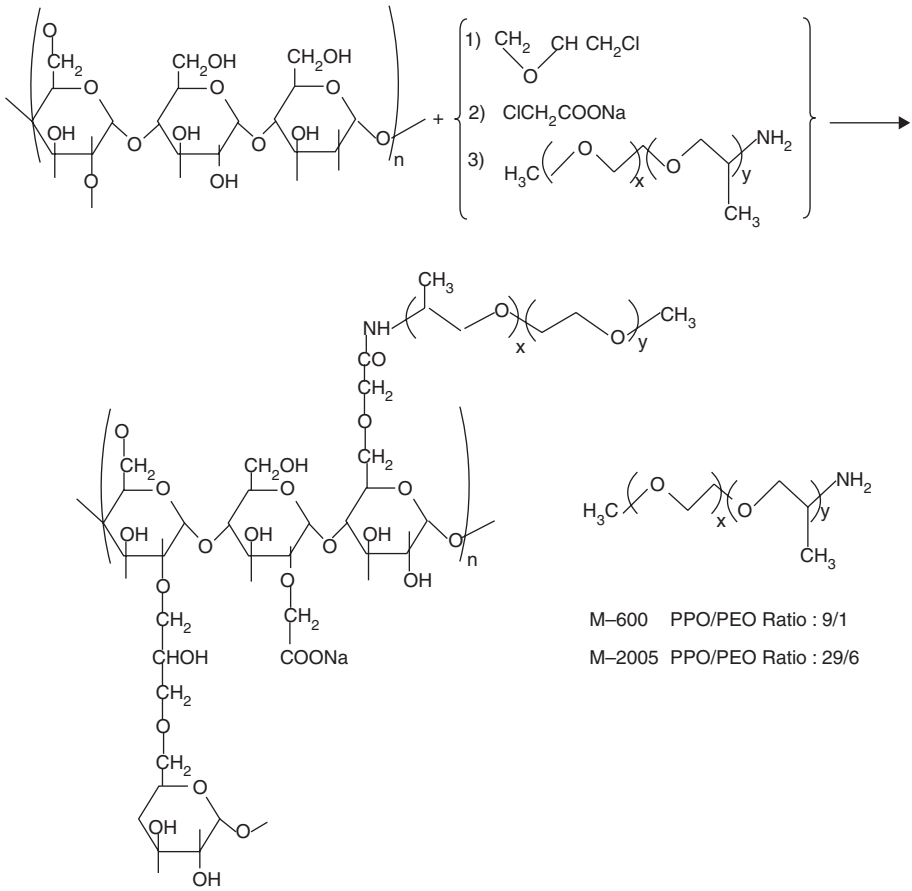
sensing groups. Ehrick *et al.* (2009) synthesized smart glucose-responsive hydrogels by immobilizing the glucose/galactose binding protein (GBP) within an acrylamide hydrogel network which demonstrated a dynamic response in the presence of glucose. They utilized the induced conformational change of *Escherichia coli* glucose-binding protein to trigger a mechanical action within a hydrogel network. The resultant hydrogel responded quantitatively to the glucose concentration and demonstrated glucose-gated selective molecular transport. Yin *et al.* (2010) synthesized a series of glucose-sensitive hydrogels based on glycidyl methacrylate (GMA)-modified dextran (Dex-G), ethylene glycol acrylate methacrylate (EGAMA)-modified concanavalin A (Con A–E) and poly(ethylene glycol) dimethacrylate (PEGDMA) via photopolymerization (Fig. 8.14). Michael addition reaction was used to obtain Con A–E, while the Dex-G precursor was prepared through ring-opening reaction. Glucose sensitivity was influenced by the content of the various components present, with PEGDMA content being the most relevant parameter. The results indicated that the hydrogels showed glucose-sensitive properties and a good biocompatibility. It has been reported that hydrogels obtained from dextran and Con A have the ability to change in response to different glucose concentrations in the environment, owing to the reversible, specific lectin–saccharide binding property (Yin *et al.*, 2010).

8.2.7 Multi-responsive smart hydrogels

Multi-responsive smart hydrogels offer responsiveness to two or more external stimuli. This enables the manipulation of a hydrogel system to achieve better targeting and efficacy in complicated microenvironments or when aiming at other functions (Dumitriu *et al.*, 2011; Guenther *et al.*, 2007; Wang *et al.*, 2010; Zhang *et al.*, 2011). Dual sensitivity was reported for a novel triblock



8.15 PAA-PEG-PAA triblock copolymer hydrogel.



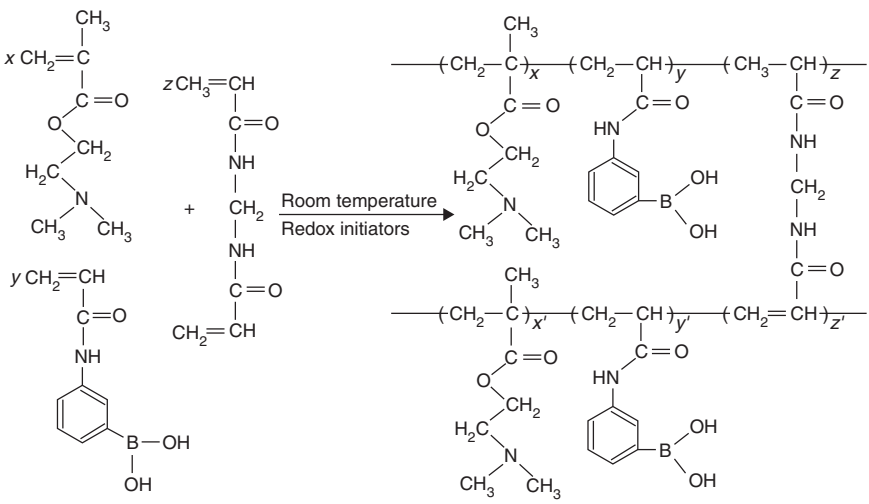
8.16 Synthesis of multi-responsive carboxymethylpullulan Jeffamine hydrogel.

copolymer poly(amidoamine)-poly(ethylene glycol)-poly(amidoamine) (PAA-PEG-PAA) by conjugating PAA to PEG via Michael addition polymerization (Fig. 8.15) (Nguyen *et al.*, 2009). The PAA block acts as a pH- and temperature-sensitive block. After injection into a rat, the copolymer solution (12.5 wt%) was immediately transformed into a gel. In addition, the hydrogels showed degradability and lack of cytotoxicity.

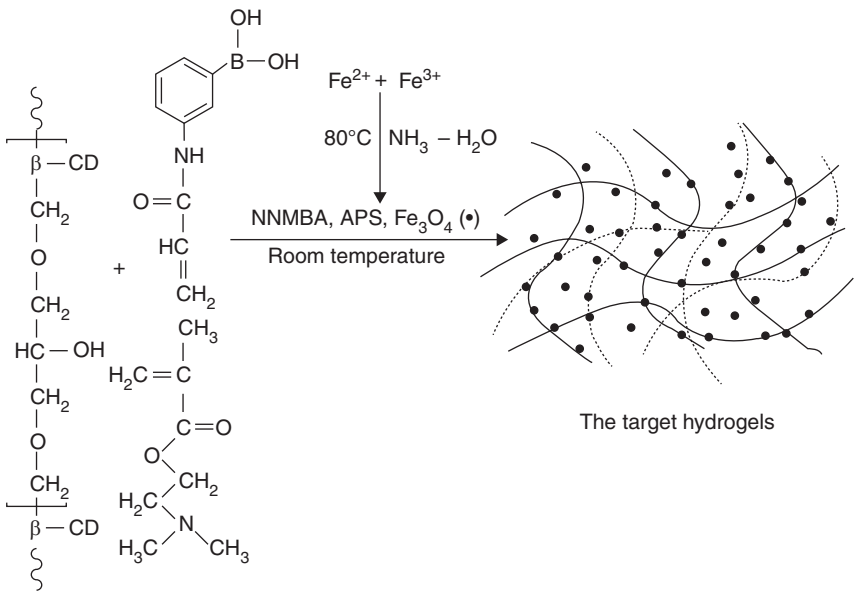
Mocanu *et al*. (2012) synthesized a multi-responsive cross-linked carboxymethylpullulan hydrogel containing Jeffamine (M-600 and M-2005) (i.e., polyoxyalkyleneamines including polyethylene oxide and polypropylene oxide based) moieties as side chains, linked via amide bonds (Fig. 8.16). Interestingly, these hydrogels showed pH-responsive properties due to the presence of anionic carboxymethyl functional groups and thermo-associative properties due to the Jeffamine moieties present. The interaction with biomolecules including antioxidants and proteins demonstrates their potential for use as controlled drug release systems (Mocanu *et al*., 2012).

Wang *et al*. (2010) developed triple-responsive hydrogels by copolymerization of (2-dimethylamino) ethyl methacrylate (DMAEMA) and glucose-sensitive moieties (i.e., 3-acrylamidephenylboronic acid, AAPBA) (Fig. 8.17). The results obtained revealed that the hydrogels displayed definite glucose sensitivity under physiological conditions, as well as sharp changes in the mesh size of their network as a function of pH and temperature changes. These multi-responsive hydrogels are highly attractive in terms of self-regulated drug delivery, as well as in other applications including actuators, regulators and separation systems possessing sensitivity to glycol.

Huang *et al*. (2012) prepared a triple-responsive Fe_3O_4/poly(3-acrylamidephenylboronic acid-co-2-(dimethylamino)ethylmethacrylate)/(β-cyclodextrin-epichlorohydrin) (Fe_3O_4/P(AAPBA-co-DMAEMA)/(β-CD-EPI)) semi-IPN (interpenetrating network) hydrogel (Fig. 8.18) by free-radical polymerization of AAPBA, DMAEMA, β-CD-EPI and the



8.17 Synthesis of triple-responsive P(DMAEMA-co-AAPBA)-based hydrogels.



8.18 Synthesis of multi-responsive Fe_3O_4/P(AAPBA-co-DMAEMA)/(β-CD-EPI) semi-IPN hydrogels.

cross-linker N,N-methylene bisacrylamide (NNMBA) in the presence of magnetite nanoparticles. The hydrogels possessed a high drug-loading efficiency for hydrophobic drugs due to the presence of cyclodextrin (CD). The semi-IPNs provided a higher mechanical strength compared to the homopolymer networks. The magnetic and multi-responsive properties of the present semi-IPN hydrogels can realize high drug-loading and magnetic targeting delivery applications (Huang *et al.*, 2012).

8.3 Applications of smart polymer hydrogels

The versatility and potential of smart hydrogel systems make them one of the most exciting interfaces of chemistry and biology systems for various biomedical applications. This section of the chapter focuses on the various applications of smart polymer hydrogels in the field of tissue engineering, drug delivery, gene delivery and protein delivery.

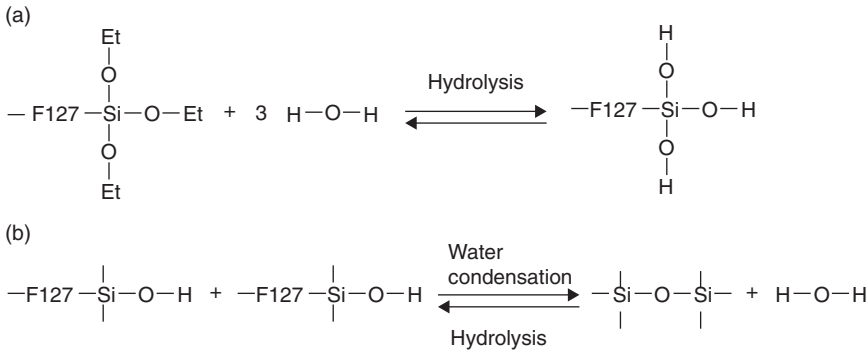
8.3.1 Tissue engineering applications

Tissue engineering aims at the replacement of damaged or diseased tissues or organs to enable the body to develop or regenerate new functional tissue. This is usually achieved through constructs containing living cells, a three-

dimensional porous matrix or scaffold, and bioactive molecules. The constructs thus support cell attachment, proliferation and differentiation. The scaffold material applied should possess properties including biocompatibility, biodegradability, mechanical strength, porosity, potential of entrapment and release of biologically active biomolecules, and an easy processability for the clinician. Hydrogels can meet all of the aforementioned properties by careful selection of the chemistry. In the field of tissue engineering, pioneering work on smart hydrogels was performed by Chung Takei Okano group using PNIPAAm and Cohn *et al.* through the application of pluronics (Chung *et al*., 1998, 1999; Takei *et al*., 1994; Cohn *et al*., 2003, 2004, 2006). Interestingly, various cells, including hepatocytes, endothelial cells, fibroblasts, keratinocytes, epithelial cells, macrophages and microglial cells, adhere and proliferate on such surfaces. Chitosan–PNIPAAm copolymer gels have been used as thermo-responsive injectable nanogels functioning as scaffolds for tissue engineering (Chen and Cheng, 2006). Interestingly, mesenchymal stem cells embedded in the copolymer solution were able to differentiate *in vitro* into chondrocytes (i.e., cells found in cartilaginous matrix). The cell–polymer mixture was injected into rabbit bladders, and the formation of new cartilage onto the polymer matrix was observed (Cho *et al*., 2004). Even though temperature-responsive surfaces based on PNIPAAm are among the most studied, other stimuli have also been investigated for tissue engineering applications including light and electrical signals. The use of electro-responsive surfaces based on conductive polypyrrole was explored, and both PC-12 as well as chicken sciatic nerve explants were shown to grow and proliferate preferentially on polypyrrole surfaces when subjected to an electric stimulus (Schmidt *et al*., 1997).

Cohn *et al*. (2005) modified Pluronics (F127) by end-capping with triethoxysilane or methacrylate reactive groups. While the methacrylates cross-linked rapidly, the triethoxysilane groups allowed the system to cross-link gradually over time. Thermo-responsive systems displaying gradually increasing mechanical properties were generated by cross-linking triethoxysilane-capped pluronics. Within time, the ethoxysilane groups hydrolyzed and resulted in the formation of silanol moieties which subsequently condensed (Fig. 8.19). In order to further improve their mechanical behavior, F127 triblocks were reacted with methacryloyl chloride and the resulting dimethacrylate was subsequently cross-linked in an aqueous solution at 37°C. The reverse thermo-responsive hydrogels of F127 dimethacrylate, demonstrated improved mechanical properties and allowed the engineering of robust macroscopic constructs, such as large tubular structures.

As an alternative, Pluronic F127 has also been modified with maleimide groups followed by subsequent reaction with the tripeptide Arg-Gly-Asp (RGD) containing peptides. The peptide-modified, thermo-responsive hydrogels were hydrolytically stable and presented an attractive material



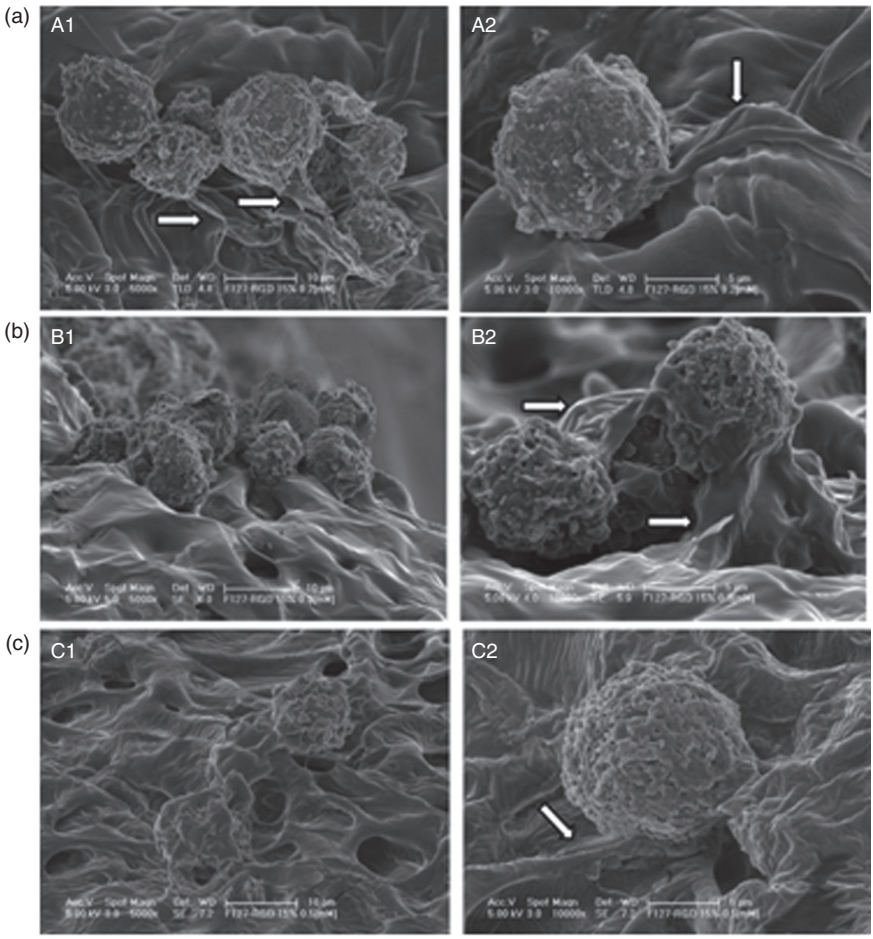
8.19 Hydrolysis of triethoxysilane end-capped pluronics (a) and subsequent condensation (b).

for tissue engineering purposes. The materials developed enabled a minimally invasive approach and supported cell survival and viability (Garty *et al*., 2010). Both the hydrogel porosity as well as its rheological properties depended on the polymer concentration applied. Interestingly, a 15% (w/v) polymer appeared to be an optimal support for cell growth, providing a structure strong enough for cell attachment. Tetracycline (Tet)-controlled expression system (Tet-Off) bone morphogenetic protein (BMP2) cells were cultured for a week in 15% (w/v) hydrogels possessing a peptide concentration of 0.2–0.6 mM. Cell-to-matrix attachment was observed at a peptide concentration of 0.2 mM (Fig. 8.20). A slight increase in peptide concentration to 0.3 mM resulted in cellular interactions and affinity of the cells for the polymer matrix (Fig. 8.20). At increasing peptide concentrations up to 0.5 mM, the cells present showed multiple interactions within the hydrogel, suggesting optimal cell–matrix interactions (Fig. 8.20).

Hybrid hydrogels based on chitosan and Pluronics have also shown thermo-responsive properties and exhibited superior hemostatic properties (Chung *et al*., 2005). Microfluidics were used by Wei *et al*. (2011) to prepare pH-responsive microcapsules based on PDMAEMA. The cationic microcapsules obtained exhibited pH-sensitivity, and the preparation conditions significantly affected their pH-responsive swelling.

8.3.2 Drug and gene delivery applications

Stimuli-sensitive hydrogels, which are able to modify their properties in response to changes in different physiological variables, are receiving increasing attention as components of therapeutic devices. In particular, polymers possessing LCST behavior or with ionizable groups which provide networks that undergo reversible phase transitions show tremendous



8.20 Scanning electron microscopy (SEM) images of Tet-off BMP2 MSCs and the peptide-modified pluronic hydrogel. The peptide concentrations include (a) 0.2 mM, A1 (5000×), A2 (10 000×); (b) 0.3 mM, B1 (5000×), B2 (10 000×); (c) 0.5 mM, C1 (5000×), C2 (10 000×).

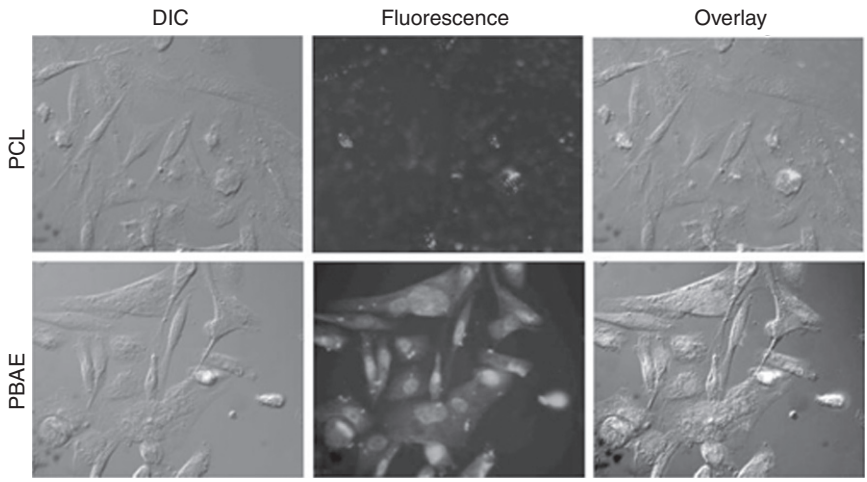
potential to develop drug delivery systems, useful to control both the drug release rate as well as its location. Lee *et al*. (2008) prepared temperature-sensitive PEI-pluronic nanocapsules by an interfacial cross-linking reaction between pre-activated Pluronic F127 and low M_W PEI using an oil-in-water interface during a modified emulsification/solvent evaporation process. Green fluorescent protein (GFP) or vascular endothelial growth factor (VEGF) siRNA, was conjugated to PEG via a disulfide linkage to form nanoscale complexes with PEI-pluronic nanocapsules. A brief cold shock to the transfected cells led to a rapid volume expansion of the nanocapsules

which could burst out an endosome compartment, enabling the siRNA cargo to be delivered into the cytosol region in a controlled manner, and subsequently silence a target mRNA.

Dadsetan *et al*. (2010) used oligo(poly(ethylene glycol) fumarate) hydrogels modified by small negatively charged sodium methacrylate for doxorubicin delivery. Sodium methacrylate at different concentrations was incorporated into the hydrogel using a photo-cross-linking method. The resulting hydrogels exhibited sensitivity to the pH and the ionic strength of the surrounding environment. The results revealed that doxorubicin was bound to the negatively charged hydrogel through electrostatic interactions and was released in a timely fashion with an ion-exchange mechanism.

Shenoy *et al*. (2005) created pH-sensitive nanoparticles capable of promoting the delivery of the anticancer drug paclitaxel to tumor cells. Poly(β-amino ester) (PBAE) with biodegradable and pH-sensitive properties was applied to formulate this delivery system. Pluronic® F-108, was mixed with PBAE to induce surface modification of the nanoparticles developed. The results indicated that the nanoparticle–paclitaxel formulation was more effective at killing the cancer cells in comparison with the free drug. To demonstrate the rapid intracellular disintegration of the PBAE nanoparticles, fluorescein isothiocyanate (FITC) was encapsulated within polymeric PBAE and poly(ε-caprolactone) (PCL) nanoparticles and incubated with the tumor cells for different time periods. Modified PCL nanoparticles served as a non-pH-responsive control. The cell images obtained after 1 h of incubation with the respective formulations indicated that the PCL nanoparticles maintained their structural integrity, and the fluorescence was limited to the nanoparticles entity only. However, the cells exposed to the PBAE nanoparticles showed diffused fluorescence within the entire cell (Fig. 8.21). After 1 h, only a small number of intact PBAE nanoparticles were present within the cellular components.

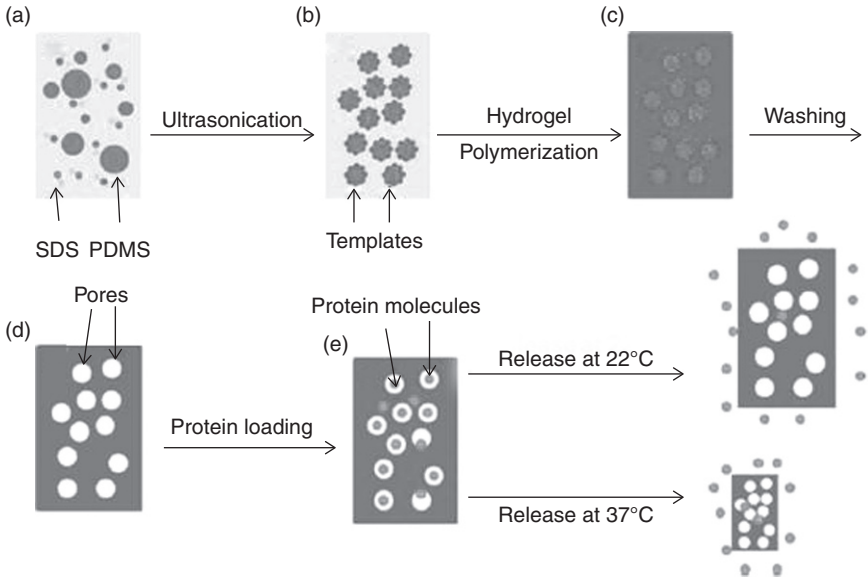
By introducing a light-sensitive chromophore (e.g., trisodium salt of copper chlorophyllin) to PNIPAM hydrogels, visible light-responsive hydrogels were prepared (Suzuki and Tanaka, 1990). When light is applied to the hydrogel, the chromophore absorbs the light which is dissipated locally as heat by radiationless transitions, increasing the 'local' temperature of the hydrogel. Interestingly, this temperature increase alters the hydrogel swelling behavior. By addition of another functional group, such as an ionizable group of PAA, the light-responsive hydrogel was also rendered pH-sensitive and then activated (i.e., induced to shrink) by visible light and deactivated (i.e., induced to swell) upon increasing the pH (Suzuki *et al*., 1996). Peppas and co-workers prepared anionic, pH-sensitive hydrogels for calcitonin entrapment (Kim *et al*., 2003). The proteins were released upon a thermal stimulus because of their physical entrapment.



8.21 Microscopic evidence for pH-sensitive triggered intracellular release of the payload from Pluronic-modified PBAE nanoparticles. F-108-modified poly(ε-caprolactone) (PCL) nanoparticles served as a non-pH-responsive control. Fluorescently-labeled modified PBAE or PCL nanoparticles were added to MDA-MB-231 human breast adenocarcinoma cells at 37°C. The first column shows the images from differential interference contrast microscopy.

8.3.3 Protein delivery applications

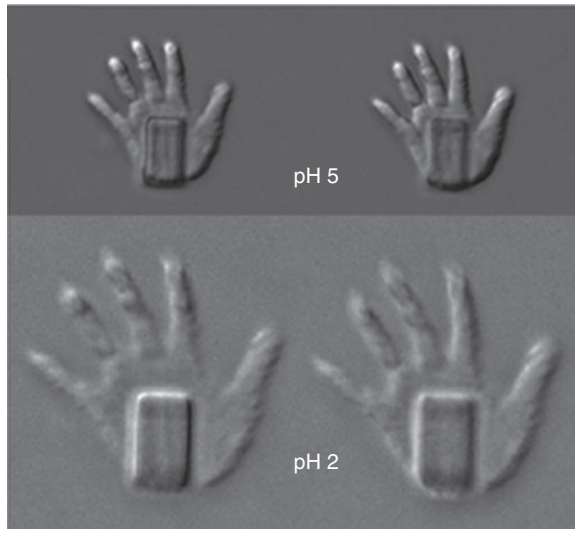
Protein release from hydrogels is very challenging, as these biomacromolecules are often fragile and possess net charges. Thus, they need to be shielded from potentially harmful species in the body. *In situ* forming polymer gels present an interesting class of stimuli-responsive polymers having great potential in protein delivery. PEG and poly(β-amino ester urethane) (PAEU) copolymers undergo pH- and temperature-induced gelation under physiological conditions (Huynh *et al*., 2011). These materials have already been used to deliver human growth hormone (hGH) to rats. The results indicated that the hGH concentration in rat serum was maintained at a higher level compared to the control, due to the controlled release rate obtained with the gel. Zhang *et al*. (2010) prepared micro-structured, temperature-sensitive PNIPAAm hydrogels and utilized these porous hydrogels as matrices for controlled protein release. The porous hydrogels were prepared using liquid poly(dimethyl siloxane) (PDMS) droplets and sodium dodecyl sulfate (SDS) as templates and stabilizer, respectively (Fig. 8.22). Two model proteins including bovine serum albumin (BSA) and lysozyme were loaded and the results indicated that lysozyme could be loaded at higher concentrations because of its molecular weight. The release kinetics of the loaded proteins was temperature-dependent and the modulated release was achieved



8.22 (a–e) Schematic illustration of preparation of highly porous smart PNIPAAm hydrogels and their application for protein release.

by adjusting the temperature. Interestingly, lysozyme and BSA kept their native folded conformation after loading and release.

Kaehr and co-workers described multiphoton fabrication of microscopic 3D hydrogels composed of proteins and demonstrated their capabilities as chemically responsive micromechanical elements. Various proteins (e.g., BSA, avidin, lysozyme) have already been used to construct materials with distinct swelling characteristics and have been combined in various ratios to tune hydrogel responsiveness (Fig. 8.23). The feasibility of modulating a swelling response was also performed by the introduction of a ligand (biotin) that stabilizes the protein (avidin) against denaturation (Kaehr and Shear, 2008). A pH-sensitive alginate–guar gum hydrogel cross-linked with glutaraldehyde has been investigated for the controlled delivery of both proteins as well as peptides. The polymers developed could swell to a minimum in the stomach, and swelling increases as the hydrogels pass through the intestinal tract where the pH is high (George and Abraham, 2007). The release profile of a model protein drug (BSA) from the hydrogels was studied under simulated gastric and intestinal media. The protein release was minimal at pH 1.2 (~20%), and it was found to be significantly higher (~90%) at pH 7.4. Prolastins, a type of silk-elastin-like protein compositions, also showed gelation properties in physiological aqueous solutions. These gels have already been used to deliver Pantarin® in a controlled manner over 24 h (Hart and Gehrke, 2007).



8.23 BSA 'microhands' mounted on BSA pedestals ~ 4 μm from the coverslip surface (upper) undergo reversible swelling after a pH decrease ranging from 5 to 2 (lower). (*Source*: Adapted with permission from Kaehr and Shear, © 2008 by The National Academy of Sciences of the USA.)

8.4 Conclusions and future trends

The present chapter has described recent progress in stimuli-responsive polymers. Temperature-, pH-, chemical- and light-sensitive polymeric hydrogels offer a very exciting field of research, not only from the basic molecular designer viewpoint but also from the perspective of biomedical applications. Stimuli-responsiveness represents a key property in medical applications because it enables a controllable response from biological compartments, such as the release of an encapsulated/entrapped active compound, the triggering of a signaling process or the detection of a specific biomolecule. Certain environmental factors such as low pH and elevated temperatures are observed in physiological conditions, due to which either pH- and/or temperature-sensitive hydrogels can be used for site-specific controlled drug delivery. Hydrogels that are responsive to specific molecules such as glucose or antigens can be employed both as biosensors and drug delivery systems. Light-sensitive, pressure-responsive and electro-sensitive hydrogels also have the potential to be used in drug delivery and bioseparation. An important aspect to be considered while developing these smart hydrogels is controlled biodegradability and biocompatibility. Research into stimuli-responsive polymers as a means of achieving this is steadily gaining momentum, and more novel polymers are being synthesized. Synthesis of

new polymers and cross-linkers with improved biocompatibility and biodegradability combined with the desired mechanical properties are essential to enable successful applications.

8.5 References

Ahn, S.-K., Kasi, R. M., Kim, S.-C., Sharma, N. and Zhou, Y. (2008). Stimuli-responsive polymer gels. *Soft Matter*, **4**, 1151–1157.

Alvarez-Lorenzo, C., Bromberg, L. and Concheiro, A. (2009). Light-sensitive intelligent drug delivery systems. *Photochemistry and Photobiology*, **85**, 848–860.

Bajpai, A. K., Shukla, S. K., Bhanu, S. and Kankane, S. (2008). Responsive polymers in controlled drug delivery. *Progress in Polymer Science*, **33**, 1088–1118.

Bromberg, L. (2001). Synthesis and self-assembly of poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide)-g-poly(acrylic acid) gels. *Industrial and Engineering Chemistry Research*, **40**, 2437–2444.

Bromberg, L. E. and Ron, E. S. (1998). Temperature-responsive gels and thermogelling polymer matrices for protein and peptide delivery. *Advanced Drug Delivery Reviews*, **31**, 197–221.

Bünsow, J. and Johannsmann, D. (2008). Electrochemically produced responsive hydrogel films: Influence of added salt on thickness and morphology. *Journal of Colloid and Interface Science*, **326**, 61–65.

Chen, J.-P. and Cheng, T.-H. (2006). Thermo-responsive chitosan-graft-poly(N-isopropylacrylamide) injectable hydrogel for cultivation of chondrocytes and meniscus cells. *Macromolecular Bioscience*, **6**, 1026–1039.

Chen, X., Li, W., Zhong, W., Lu, Y. and Yu, T. (1997). pH sensitivity and ion sensitivity of hydrogels based on complex-forming chitosan/silk fibroin interpenetrating polymer network. *Journal of Applied Polymer Science*, **65**, 2257–2262.

Cho, J. H., Kim, S.-H., Park, K. D., Jung, M. C., Yang, W. I., Han, S. W., Noh, J. Y. and Lee, J. W. (2004). Chondrogenic differentiation of human mesenchymal stem cells using a thermosensitive poly(N-isopropylacrylamide) and water-soluble chitosan copolymer. *Biomaterials*, **25**, 5743–5751.

Chung, H. J., Bae, J. W., Park, H. D., Lee, J. W. and Park, K. D. (2005). Thermosensitive chitosans as novel injectable biomaterials. *Macromolecular Symposia*, **224**, 275–286.

Chung, J. E., Yokoyama, M., Aoyagi, T., Sakurai, Y. and Okano, T. (1998). Effect of molecular architecture of hydrophobically modified poly(N-isopropylacrylamide) on the formation of thermoresponsive core-shell micellar drug carriers. *Journal of Controlled Release*, **53**, 119–130.

Chung, J. E., Yokoyama, M., Yamato, M., Aoyagi, T., Sakurai, Y. and Okano, T. (1999). Thermo-responsive drug delivery from polymeric micelles constructed using block copolymers of poly(N-isopropylacrylamide) and poly(butylmethacrylate). *Journal of Controlled Release*, **62**, 115–127.

Cohn, D., Lando, G., Sosnik, A., Garty, S. and Levi, A. (2006). PEO–PPO–PEO-based poly(ether ester urethane)s as degradable reverse thermo-responsive multiblock copolymers. *Biomaterials*, **27**, 1718–1727.

Cohn, D., Sosnik, A. and Garty, S. (2005). Smart hydrogels for in situ generated implants. *Biomacromolecules*, **6**, 1168–1175.

Cohn, D., Sosnik, A. and Levy, A. (2003). Improved reverse thermo-responsive polymeric systems. *Biomaterials*, **24**, 3707–3714.

Cushing, M. C. and Anseth, K. S. (2007). Hydrogel cell cultures. *Science*, **316**, 1133–1134.

Dadsetan, M., Liu, Z., Pumberger, M., Giraldo, C. V., Ruesink, T., Lu, L. and Yaszemski, M. J. (2010). A stimuli-responsive hydrogel for doxorubicin delivery. *Biomaterials*, **31**, 8051–8062.

Denet, A.-R., Vanbever, R. and Préat, V. (2004). Skin electroporation for transdermal and topical delivery. *Advanced Drug Delivery Reviews*, **56**, 659–674.

Deng, L., Zhai, Y., Lin, X., Jin, F., He, X. and Dong, A. (2008). Investigation on properties of re-dispersible cationic hydrogel nanoparticles. *European Polymer Journal*, **44**, 978–986.

Dou, X.-Q., Yang, X.-M., Li, P., Zhang, Z.-G., Schonherr, H., Zhang, D. and Feng, C.-L. (2012). Novel pH responsive hydrogels for controlled cell adhesion and triggered surface detachment. *Soft Matter*, **8**, 9539–9544.

Dumitriu, R. P., Mitchell, G. R. and Vasile, C. (2011). Multi-responsive hydrogels based on N-isopropylacrylamide and sodium alginate. *Polymer International*, **60**, 222–233.

Ehrick, J. D., Luckett, M. R., Khatwani, S., Wei, Y., Deo, S. K., Bachas, L. G. and Daunert, S. (2009). Glucose responsive hydrogel networks based on protein recognition. *Macromolecular Bioscience*, **9**, 864–868.

Fernandez-Tarrio, M., Yañez, F., Immesoete, K., Alvarez-Lorenzo, C. and Concheiro, A. (2008). Pluronic and tetronic copolymers with polyglycolyzed oils as self-emulsifying drug delivery systems. *AAPS PharmSciTech*, **9**, 471–479.

Garty, S., Kimelman-Bleich, N., Hayouka, Z., Cohn, D., Friedler, A., Pelled, G. and Gazit, D. (2010). Peptide-modified 'smart' hydrogels and genetically engineered stem cells for skeletal tissue engineering. *Biomacromolecules*, **11**, 1516–1526.

George, M. and Abraham, T. E. (2007). pH sensitive alginate–guar gum hydrogel for the controlled delivery of protein drugs. *International Journal of Pharmaceutics*, **335**, 123–129.

Griffin, D. R. and Kasko, A. M. (2012). Photodegradable macromers and hydrogels for live cell encapsulation and release. *Journal of the American Chemical Society*, **134**, 13103–13107.

Guenther, M., Kuckling, D., Corten, C., Gerlach, G., Sorber, J., Suchaneck, G. and Arndt, K. F. (2007). Chemical sensors based on multiresponsive block copolymer hydrogels. *Sensors and Actuators B: Chemical*, **126**, 97–106.

Hamcerencu, M., Desbrieres, J., Popa, M. and Riess, G. R. (2009). Stimuli-sensitive xanthan derivatives/N-isopropylacrylamide hydrogels: influence of cross-linking agent on interpenetrating polymer network properties. *Biomacromolecules*, **10**, 1911–1922.

Hart, D. S. and Gehrke, S. H. (2007). Thermally associating polypeptides designed for drug delivery produced by genetically engineered cells. *Journal of Pharmaceutical Sciences*, **96**, 484–516.

Hassouneh, W., Macewan, S. R. and Chilkoti, A. (2012). Chapter nine – fusions of elastin-like polypeptides to pharmaceutical proteins. *In*: Wittrup, K. D. and Gregory, L. V. (eds.) *Methods in Enzymology*. Academic Press.

He, L., Fullenkamp, D. E., Rivera, J. G. and Messersmith, P. B. (2011). pH responsive self-healing hydrogels formed by boronate-catechol complexation. *Chemical Communications*, **47**, 7497–7499.

Huang, Y., Liu, M., Chen, J., Gao, C. and Gong, Q. (2012). A novel magnetic triple-responsive composite semi-IPN hydrogels for targeted and controlled drug delivery. *European Polymer Journal*, **48**, 1734–1744.

Huber, S., Hutter, N. and Jordan, R. (2008). Effect of end group polarity upon the lower critical solution temperature of poly(2-isopropyl-2-oxazoline). *Colloid and Polymer Science*, **286**, 1653–1661.

Huynh, C. T., Kang, S. W., Li, Y., Kim, B. S. and Lee, D. S. (2011). Controlled release of human growth hormone from a biodegradable pH/temperature-sensitive hydrogel system. *Soft Matter*, **7**, 8984–8990.

Ilg, P. (2013). Stimuli-responsive hydrogels cross-linked by magnetic nanoparticles. *Soft Matter*, **9**, 3465–3468.

Ishida, K., Uno, T., Itoh, T. and Kubo, M. (2012). Synthesis and property of temperature-responsive hydrogel with movable cross-linking points. *Macromolecules*, **45**, 6136–6142.

Jain, R., Standley, S. M. and Fréchet, J. M. J. (2007). Synthesis and degradation of pH-sensitive linear poly(amidoamine)s. *Macromolecules*, **40**, 452–457.

Jensen, M., Birch Hansen, P., Murdan, S., Frokjaer, S. and Florence, A. T. (2002). Loading into and electro-stimulated release of peptides and proteins from chondroitin 4-sulphate hydrogels. *European Journal of Pharmaceutical Sciences*, **15**, 139–148.

Kaehr, B. and Shear, J. B. (2008). Multiphoton fabrication of chemically responsive protein hydrogels for microactuation. *Proceedings of the National Academy of Sciences*, **105**, 8850–8854.

Kim, B., LA Flamme, K. and Peppas, N. A. (2003). Dynamic swelling behavior of pH-sensitive anionic hydrogels used for protein delivery. *Journal of Applied Polymer Science*, **89**, 1606–1613.

Kim, J. J. and Park, K. (2001). Modulated insulin delivery from glucose-sensitive hydrogel dosage forms. *Journal of Controlled Release*, **77**, 39–47.

Kopecek, J. (2002). Polymer chemistry – Swell gels. *Nature*, **417**, 388–391.

Kopecek, J. (2009). Hydrogels: From soft contact lenses and implants to self-assembled nanomaterials. *Journal of Polymer Science Part A: Polymer Chemistry*, **47**, 5929–5946.

Krogsgaard, M., Behrens, M. A., Pedersen, J. S. and Birkedal, H. (2013). Self-healing mussel-inspired multi-pH-responsive hydrogels. *Biomacromolecules*, **14**, 297–301.

Kulkarni, R. V. and Sa, B. (2009). Electroresponsive polyacrylamide-grafted-xanthan hydrogels for drug delivery. *Journal of Bioactive and Compatible Polymers*, **24**, 368–384.

Kumar, A., Srivastava, A., Galaev, I. Y. and Mattiasson, B. (2007). Smart polymers: Physical forms and bioengineering applications. *Progress in Polymer Science*, **32**, 1205–1237.

Lee, S. H., Choi, S. H., Kim, S. H. and Park, T. G. (2008). Thermally sensitive cationic polymer nanocapsules for specific cytosolic delivery and efficient gene silencing of siRNA: Swelling induced physical disruption of endosome by cold shock. *Journal of Controlled Release*, **125**, 25–32.

Liu, X.-W., Zhu, S., Wu, S.-R., Wang, P. and Han, G.-Z. (2013). Response behavior of ion-sensitive hydrogel based on crown ether. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **417**, 140–145.

Mabrouk, E., Cuvelier, D., Brochard-Wyart, F., Nassoy, P. and Li, M.-H. (2009). Bursting of sensitive polymersomes induced by curling. *Proceedings of the National Academy of Sciences*, **106**, 7294–7298.

Messing, R. and Schmidt, A. M. (2011). Perspectives for the mechanical manipulation of hybrid hydrogels. *Polymer Chemistry*, **2**, 18–32.

Miyata, T., Nakamae, K., Hoffman, A. S. and Kanzaki, Y. (1994). Stimuli-sensitivities of hydrogels containing phosphate groups. *Macromolecular Chemistry and Physic*s, **195**, 1111–1120.

Mocanu, G., Souguir, Z., Picton, L. and Le Cerf, D. (2012). Multi-responsive carboxymethyl polysaccharide crosslinked hydrogels containing Jeffamine sidechains. *Carbohydrate Polymers*, **89**, 578–585.

Murdan, S. (2003). Electro-responsive drug delivery from hydrogels. *Journal of Controlled Release*, **92**, 1–17.

Nakamae, K., Miyata, T. and Hoffman, A. S. (1992). Swelling behavior of hydrogels containing phosphate groups. *Die Makromolekulare Chemie*, **193**, 983–990.

Nguyen, M. K., Park, D. K. and Lee, D. S. (2009). Injectable poly(amidoamine)-poly(ethylene glycol)-poly(amidoamine) triblock copolymer hydrogel with dual sensitivities: pH and temperature. *Biomacromolecules*, **10**, 728–731.

Osada, Y. and Hasebe, M. (1985). Electrically activated mechanochemical devices using polyelectrolyte gels. *Chemistry Letters*, **14**, 1285–1288.

Peppas, N. A., Hilt, J. Z., Khademhosseini, A. and Langer, R. (2006). Hydrogels in biology and medicine: from molecular principles to bionanotechnology. *Advanced Materials*, **18**, 1345–1360.

Prabaharan, M. and Mano, J. F. (2006). Stimuli-responsive hydrogels based on polysaccharides incorporated with thermo-responsive polymers as novel biomaterials. *Macromolecular Bioscience*, **6**, 991–1008.

Qiu, Y. and Park, K. (2001). Environment-sensitive hydrogels for drug delivery. *Advanced Drug Delivery Reviews*, **53**, 321–339.

Rahimi, N., Molin, D. G., Cleij, T. J., VAN Zandvoort, M. A. and Post, M. J. (2012). Electrosensitive polyacrylic acid/fibrin hydrogel facilitates cell seeding and alignment. *Biomacromolecules*, **13**, 1448–1457.

Ramanathan, S. and Block, L. H. (2001). The use of chitosan gels as matrices for electrically-modulated drug delivery. *Journal of Controlled Release*, **70**, 109–123.

Rasool, N., Yasin, T., Heng, J. Y. Y. and Akhter, Z. (2010). Synthesis and characterization of novel pH-, ionic strength and temperature- sensitive hydrogel for insulin delivery. *Polymer*, **51**, 1687–1693.

Satarkar, N. S., Biswal, D. and Hilt, J. Z. (2010). Hydrogel nanocomposites: A review of applications as remote controlled biomaterials. *Soft Matter*, **6**, 2364–2371.

Schmidt, C. E., Shastri, V. R., Vacanti, J. P. and Langer, R. (1997). Stimulation of neurite outgrowth using an electrically conducting polymer. *Proceedings of the National Academy of Sciences*, **94**, 8948–8953.

Schoener, C. A., Hutson, H. N. and Peppas, N. A. (2012). pH-responsive hydrogels with dispersed hydrophobic nanoparticles for the delivery of hydrophobic therapeutic agents. *Polymer International*, **61**, 874–879.

Shenoy, D., Little, S., Langer, R. and Amiji, M. (2005). Poly(ethylene oxide)-modified poly(β-amino ester) nanoparticles as a pH-sensitive system for tumor-targeted delivery of hydrophobic drugs. 1. in vitro evaluations. *Molecular Pharmaceutics*, **2**, 357–366.

Soppimath, K. S., Aminabhavi, T. M., Dave, A. M., Kumbar, S. G. and Rudzinski, W. E. (2002). Stimulus-responsive 'smart' hydrogels as novel drug delivery systems*. *Drug Development and Industrial Pharmacy*, **28**, 957–974.

Sosnik, A. and Cohn, D. (2004). Ethoxysilane-capped PEO–PPO–PEO triblocks: a new family of reverse thermo-responsive polymers. *Biomaterials*, **25**, 2851–2858.

Sutani, K., Kaetsu, I., Uchida, K. and Matsubara, Y. (2002). Stimulus responsive drug release from polymer gel: Controlled release of ionic drug from polyampholyte gel. *Radiation Physics and Chemistry*, **64**, 331–336.

Suzuki, A. and Tanaka, T. (1990). Phase-transition in polymer gels induced by visible-light. *Nature*, **346**, 345–347.

Suzuki, A., Ishii, T. and Maruyama, Y. (1996). Optical switching in polymer gels. *Journal of Applied Physics*, **80**, 131–136.

Takei, Y. G., Aoki, T., Sanui, K., Ogata, N., Sakurai, Y. and Okano, T. (1994). Dynamic contact angle measurement of temperature-responsive surface properties for poly(N-isopropylacrylamide) grafted surfaces. *Macromolecules*, **27**, 6163–6166.

Tanaka, T., Nishio, I., Sun, S.-T. and Ueno-Nishio, S. (1982). Collapse of gels in an electric field. *Science*, **218**, 467–469.

Traitel, T., Cohen, Y. and Kost, J. (2000). Characterization of glucose-sensitive insulin release systems in simulated in vivo conditions. *Biomaterials*, **21**, 1679–1687.

Trongsatitkul, T. and Budhlall, B. M. (2013). Microgels or microcapsules? Role of morphology on the release kinetics of thermoresponsive PNIPAm-co-PEGMa hydrogels. *Polymer Chemistry*, **4**, 1502–1516.

Velthoen, I. W., VAN Beek, J., Dijkstra, P. J. and Feijen, J. (2011). Thermo-responsive hydrogels based on highly branched poly(ethylene glycol)–poly(l-lactide) copolymers. *Reactive and Functional Polymers*, **71**, 245–253.

Wang, C., Stewart, R. J. and Kopecek, J. (1999). Hybrid hydrogels assembled from synthetic polymers and coiled-coil protein domains. *Nature*, **397**, 417–420.

Wang, K., Fu, Q., Chen, X., Gao, Y. and Dong, K. (2012). Preparation and characterization of pH-sensitive hydrogel for drug delivery system. *RSC Advances*, **2**, 7772–7780.

Wang, L., Liu, M., Gao, C., Ma, L. and Cui, D. (2010). A pH-, thermo-, and glucose-, triple-responsive hydrogels: Synthesis and controlled drug delivery. *Reactive and Functional Polymers*, **70**, 159–167.

Ward, M. A. and Georgiou, T. K. (2011). Thermoresponsive polymers for biomedical applications. *Polymers*, **3**, 1215–1242.

Wei, J., Ju, X.-J., Xie, R., Mou, C.-L., Lin, X. and Chu, L.-Y. (2011). Novel cationic pH-responsive poly(N,N-dimethylaminoethyl methacrylate) microcapsules prepared by a microfluidic technique. *Journal of Colloid and Interface Science*, **357**, 101–108.

Yan, B., Boyer, J.-C., Habault, D., Branda, N. R. and Zhao, Y. (2012). Near infrared light triggered release of biomacromolecules from hydrogels loaded with upconversion nanoparticles. *Journal of the American Chemical Society*, **134**, 16558–16561.

Yang, S., Liu, G., Wang, X. and Song, J. (2010). Electroresponsive behavior of a sulfonated poly(vinyl alcohol) hydrogel and its application to electrodriven artificial fish. *Journal of Applied Polymer Science*, **117**, 2346–2353.

Yin, R., Wang, K., Han, J. and Nie, J. (2010). Photo-crosslinked glucose-sensitive hydrogels based on methacrylate modified dextran–concanavalin A and PEG dimethacrylate. *Carbohydrate Polymers*, **82**, 412–418.

Yoshikawa, H. Y., Rossetti, F. F., Kaufmann, S., Kaindl, T., Madsen, J., Engel, U., Lewis, A. L., Armes, S. P. and Tanaka, M. (2011). Quantitative evaluation of mechanosensing of cells on dynamically tunable hydrogels. *Journal of the American Chemical Society*, **133**, 1367–1374.

Zhang, H., Yan, Q., Kang, Y., Zhou, L., Zhou, H., Yuan, J. and Wu, S. (2012). Fabrication of thermo-responsive hydrogels from star-shaped copolymer with a biocompatible β-cyclodextrin core. *Polymer*, **53**, 3719–3725.

Zhang, J.-T., Petersen, S., Thunga, M., Leipold, E., Weidisch, R., Liu, X., Fahr, A. and Jandt, K. D. (2010). Micro-structured smart hydrogels with enhanced protein loading and release efficiency. *Acta Biomaterialia*, **6**, 1297–1306.

Zhang, R., Tang, M., Bowyer, A., Eisenthal, R. and Hubble, J. (2006). Synthesis and characterization of a d-glucose sensitive hydrogel based on CM-dextran and concanavalin A. *Reactive and Functional Polymers*, **66**, 757–767.

Zhang, S.-B., Chu, L.-Y., Xu, D., Zhang, J., Ju, X.-J. and Xie, R. (2008). Poly(N-isopropylacrylamide)-based comb-type grafted hydrogel with rapid response to blood glucose concentration change at physiological temperature. *Polymers for Advanced Technologies*, **19**, 937–943.

Zhang, Y., Tao, L., Li, S. and Wei, Y. (2011). Synthesis of multiresponsive and dynamic chitosan-based hydrogels for controlled release of bioactive molecules. *Biomacromolecules*, **12**, 2894–2901.

Zhao, B. and Moore, J. S. (2001). Fast pH- and ionic strength-responsive hydrogels in microchannels. *Langmuir*, **17**, 4758–4763.

Zhao, Y.-L. and Stoddart, J. F. (2009). Azobenzene-based light-responsive hydrogel system. *Langmuir*, **25**, 8442–8446.

Zhu, C.-H., Lu, Y., Peng, J., Chen, J.-F. and Yu, S.-H. (2012). Photothermally sensitive poly(N-isopropylacrylamide)/graphene oxide nanocomposite hydrogels as remote light-controlled liquid microvalves. *Advanced Functional Materials*, **22**, 4017–4022.

9

Self-healing polymer systems: properties, synthesis and applications

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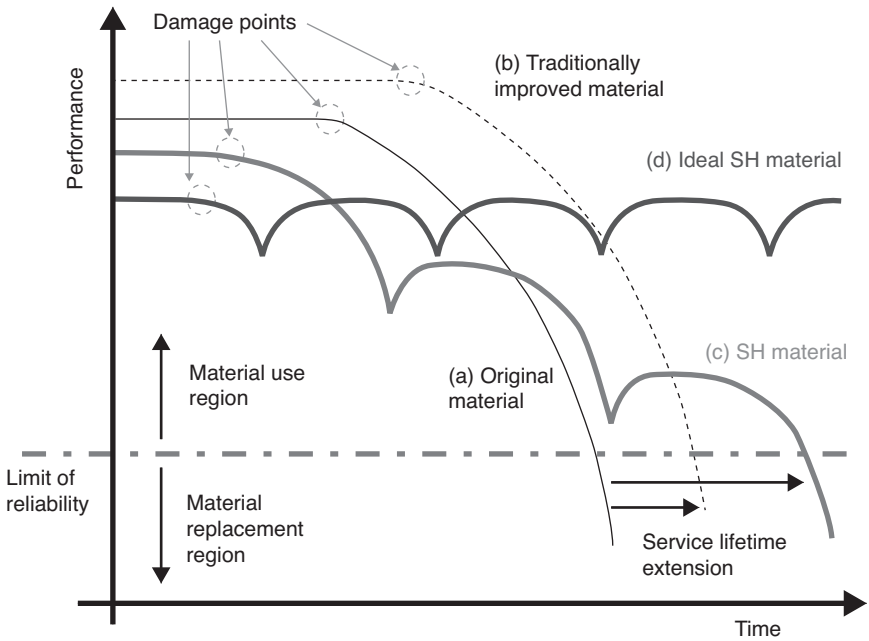
Abstract: After millions of years of evolution, nature has developed materials and systems based on the concept of damage management (healing) in order to extend survival possibilities. In the last 20 years, the dream of implementing this concept to engineering systems to extend service lifetime has become a reality. The field of self-healing engineering materials is growing exponentially both in numbers of research groups involved and concepts explored, although finding applications for many of these new concepts is not straightforward. In this chapter we focus on the latest developments in the field of self-healing polymeric systems, analysing the main mechanisms and concepts as well as possible applications.

Key words: self-healing, intrinsic healing, extrinsic healing, functionality, damage.

9.1 Introduction

9.1.1 Self-healing materials

Occurrence of damage is an unavoidable fact in man-made materials/systems as well as in natural ones. The amount of (accumulated) damage a system experiences depends on time (i.e. fatigue damage such as ageing) and/or one-time adverse events (i.e. sudden event damage such as impact or scratch) (Fischer, 2010; Garcia *et al.,* 2011a). Damage can thus be defined as undesired alterations at the molecular and/or macroscale leading to total or partial loss of an original functionality of the material. A functionality is here defined as an intrinsic property of a material that justifies the presence of the material in the system, for instance, colour, strength, adhesion, corrosion protection, brightness, hydrophobicity, wear resistance, reflectivity, electrical or thermal conductivity, stealth, ion selection, barrier or transparency.



9.1 (a–d) Self-healing concept in materials development.

As such, all material functionalities are the result of a combination of appropriate material architecture and physico-chemical characteristics of its constituent elements. Small-scale damage affecting the chemical structure or architecture will lead to a decrease of the intended properties/functionality which can further lead to the replacement of the damaged area or, in the worst case scenario, to the catastrophic failure of the structure with consequent loss of time, material and money.

A traditional approach towards longer use of structures and systems is the development of more resistant and better materials. This means materials that have an initially higher performance than the material they replace so that the material can be used for a longer time (see 'a' and 'b' in Fig. 9.1). In recent years a more dynamic strategy based on damage acceptance and management (i.e. self-repairing or self-healing material systems) has been explored and interest has grown exponentially, especially after the communication of White *et al*. in Nature in 2001 (White *et al.,* 2001).

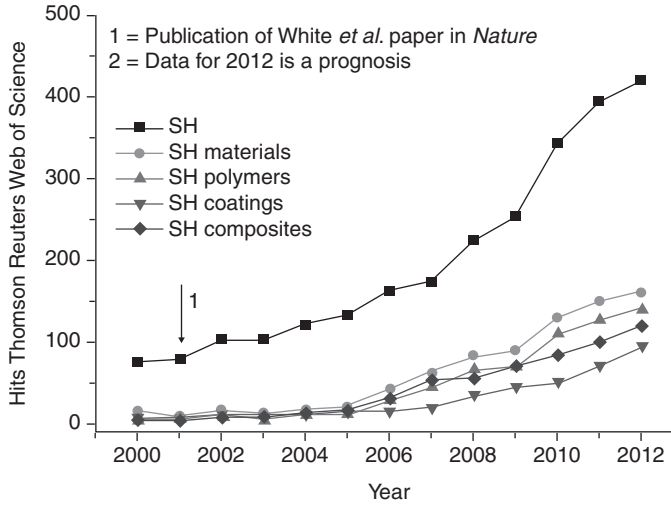
The aim of the self-healing materials concept is to extend the service lifetime of materials, structures and systems made thereof, not by increasing their initial performance but by implementing the concept of autonomous or induced repair. This new trend in materials development is based on a

damage management concept instead of on damage prevention, assuming that damage is unavoidable and that our materials have to be prepared for it (van der Zwaag, 2007). In Fig. 9.1, curve 'c' shows the intended effect of the implementation of the self-healing concept on a material. For this self-healing material the initial properties are lower than those of the original material it replaces ('a') so that damage occurs earlier; nevertheless, thanks to the implemented healing capability, the material partially restores its initial properties allowing a significant extension of its service lifetime. At the same time an ideal self-healing system would theoretically look like curve 'd', with continuous healing events that allow the recovery almost to the original level of properties, thus extending the lifetime even further.

Analysing a healing process and existing engineering approaches leads to the realization that self-healing:

- *requires* a mobile phase stored in containers or that the material itself is mobile when desired;
- *may require* externally supplied agents (e.g. H_2O, O_2);
- *requires* a resting period for the healing to take place;
- *may require* the supply of modest amounts of energy (e.g. UV, local temperature, high kinetic energy) to temporarily and locally increase the mobility necessary for healing; and
- *may require* detection and activation mechanisms to initiate healing.

In recent decades, the concept of self-healing materials has attracted significant attention. In this sense a literature search via the Thomson Reuters Web of Science can already provide a first order approximation of the actual status of the field, at least at the scientific level (Fig. 9.2). A search using the words 'self-healing' leads to a significant number of hits. Nevertheless, it should be noted that these hits do not only refer to the field of materials science, especially in its development to 2005, but also to hits including the medical and informatics fields. For the trend in self-healing materials other search terms are preferred, such as 'self-healing materials', 'composites', 'polymers' and 'coatings'. In 2001, White *et al*. (2001) published what is considered the fundamental work in the field of self-healing materials. Following that, research developments in the field required about five years of incubation time till the research field took off and it is still growing. Not surprisingly, as plotted explicitly in Fig. 9.2, the majority of papers published in self-healing materials deals with self-healing polymers since they offer the best starting conditions (molecular mobility) for the design of such systems, while application fields such as coatings (including organic and inorganic) are just taking off, as they address specific applications that make use of developments in the polymer field, amongst others. Self-healing composites



9.2 Hits per decade in the field of self-healing materials from ISI Web of Science.

may be an extension of the field of self-healing polymers showing a growth rate similar to that of self-healing polymers.

9.1.2 Classification of self-healing materials

The existing self-healing concepts applicable to bulk polymers can be classified as shown in Table 9.1 and adapted for self-healing polymeric coatings based on a critical review by Garcia *et al.* (2011a). The classification in levels as found in Table 9.1 aims to offer a benchmark for the various modes of healing approaches relevant to organic coatings and polymers as found in literature. From this table, the most general classification that will be used in the rest of this paper is the distinction given between *extrinsic* (or discontinuous) and *intrinsic* (or continuous) as opposed to traditional differentiation between autonomous and non-autonomous healing (Blaiszik *et al.*, 2010a; Williams *et al.*, 2008), as the latter classification does not consider that one healing system may be non-autonomous under certain circumstances and autonomous under other conditions. For the rest of the paper we refer to 'self-healing' or 'healing' materials as those materials able to repair themselves or restore a functionality or property with or without external help.

This chapter aims to present the most recently explored and developed concepts and applications in the field of self-healing materials, providing a comprehensible introduction to the field for the uninitiated. At the same

Table 9.1 Classification of existing self-healing concepts for polymeric systems

Concept (Level 1)	Architecture (Level 2)	Healing mechanism (Level 3)	Damage scale (Level 4)
Additive or extrinsic (discontinuous)	Containers (capsules, fibres, networks)	Liquid spreads and reacts (flow)	μm–mm (micro–macro)
	Expansive phases	Reacts and expands (expansion)	μm (micro)
	Corrosion inhibitors	Release and reacts with metallic surface (flow)	nm–μm (nano–micro)
Intrinsic (continuous)	No architecture	Reversible chemical bonds (re-flow)	nm–mm (nano–micro)
		Reversible non-covalent bonds (re-flow)	nm–mm (nano–micro)
		Delayed elasticity (re-flow)	nm–μm (nano–micro)
		Unreacted groups (re-flow)	
		Surface stratification (re-flow)	

Source: Adapted from Garcia *et al.* (2011).

time the second intention of this work is to inspire the reader to find applications to the many already developed concepts as well as to invite the reader to be open minded to new chemistries which may be applicable in self-healing systems with the final goal of healing certain functionalities.

9.2 Types of self-healing

9.2.1 Extrinsic healing

An extrinsic self-healing approach refers to all systems in which the healing agent (monomer, oligomer, solvent, etc.) is included in the system as an isolated separate phase, including all container-based approaches such as capsules, fibres and nanocarriers. This extrinsic concept offers as its main advantage a localized response when damage occurs by breaking the containers and releasing the healing agent within, which reacts in the presence of other components (such as catalysts or cross-linkers) present in the surrounding media or embedded as other extrinsic phases in the matrix. The main disadvantage of this concept is the limitations with respect to repeated healing at previously damaged and healed sites.

Spherical capsules containing healing agents

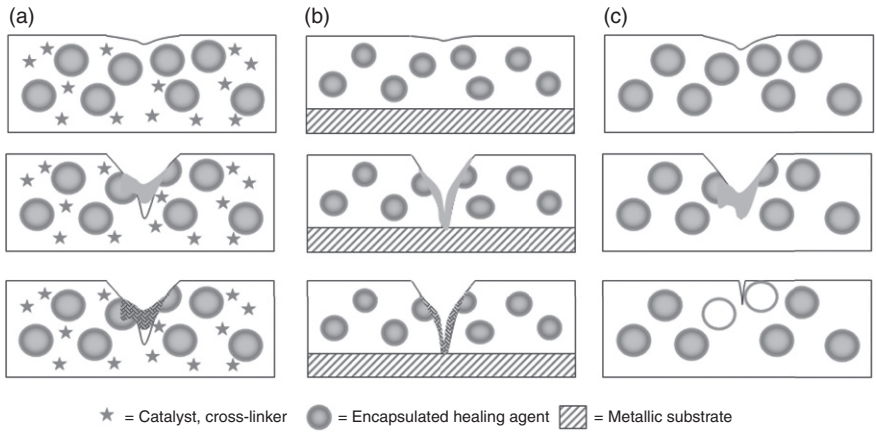
Since the encouraging paper on liquid encapsulation by White *et al*. (2001), the amount of research based on this healing route has highly advanced mainly in four directions:

1. improvement in encapsulation techniques such as higher efficiency, stronger shell walls, capsule homogeneity, capsule stability, capsule geometries – from spherical to ellipsoidal, capsule size, etc. (Mookhoek *et al.*, 2009; Nesterova *et al.*, 2011, 2012; Samadzadeh *et al.,* 2010);
2. selection of healing agent–cross-linker/catalyst pairs suitable for different matrices and encapsulating shell materials, for instance, use of efficient, less expensive catalysts, use of healing agents adapted to the medium in use, and use of new encapsulated chemistries such as azide/alkene click reactions (Gragert *et al.,* 2011) etc.;
3. development of healing agents which do not require a cross-linker or catalyst, that is, solvents (Caruso *et al.,* 2007; Mookhoek *et al.*, 2010), water and surface reactive agents such as silyl esters and oils (Garcia *et al.*, 2011b; Kumar *et al*., 2006; Samadzadeh *et al.*, 2011); and
4. implementation of the encapsulation concept to more application-oriented research.

Figure 9.3 shows the three main approaches based on embedded liquid healing agents. Firstly, there are two-agent systems in which the healing agent is encapsulated and the catalyst is dispersed in the matrix. Upon contact, the healing agent hardens and keeps the two sides of the crack together, acting as a glue. The same concept can be used for dual encapsulation systems where one capsule type contains the healing agent and the second capsule type contains the hardener. Second, there is the one agent system reacting with its environment, such as the silyl ester approach developed for corrosion protection in which a reaction takes place with the humidity and the underlying metal forming a barrier-protective surface layer. Thirdly, one agent reacts with the matrix, such as an encapsulated solvent in a thermoplastic, allowing sufficient mobility to heal damage.

Hollow fibres containing healing agents

Fibres as healing agent carriers have been used almost since the first successful attempts to demonstrate the self-healing concept in engineering materials (Dry *et al*., 1993). As for spherical capsules, the healing mechanism is based on a liquid confined in a container (fibre in this case) which, upon rupture, leads to release of the agent and subsequent reaction. The main

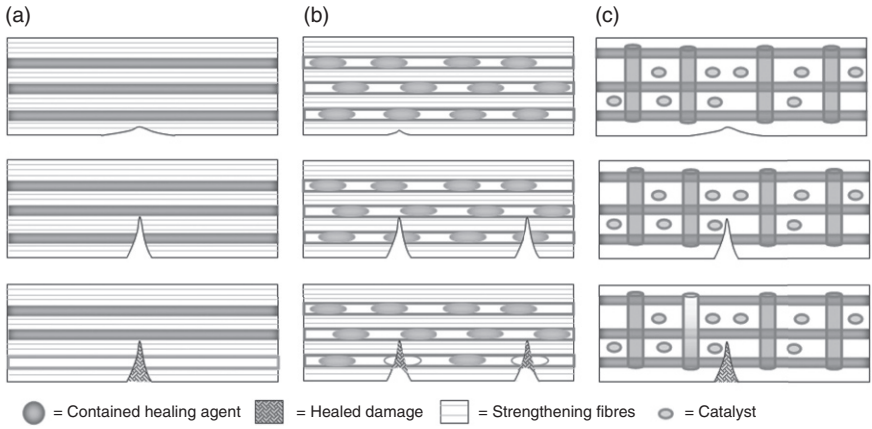


9.3 Existing concepts using the encapsulation route: (a) two component healing agent; (b) media reactive sealing component agent and (c) solvent in thermoplastic polymer.

difference between this approach and the use of dispersed spherical capsules is that in this case the probability of the healing fibre causing damage is higher. On the other hand, this approach is designed to be directly applied on fibre composites, where some of the strengthening fibres are replaced by filled fibres with the healing capability, allowing a relatively fast implementation of the concept in existing applications. Similar healing agents as for spherical capsules can be used.

Figure 9.4 shows the three existing approaches using hollow fibres: (a) hollow fibres; (b) compartmented fibres; and (c) vascular systems. Of these, the most common are individual hollow fibres (Dry *et al.,* 1993; Liu *et al*., 2008; Pang and Bond, 2005) and vascular systems (Toohey *et al.,* 2009), which are networks of fibres able to transport the healing agents, needed in a constant supply, in the way that veins function in animals. The approach using compartmented fibres (Mookhoek *et al.*, 2012) is a more recent one, still at the proof of concept stage. In this concept the healing agent is confined in containers in a fibre, thus combining the advantages of hollow fibres (high probability of break and release) and isolated spherical/elongated capsules (localized release and possibility of multiple repairs using the same structure).

Each one of the approaches has advantages and disadvantages and the final application of one or the other will depend on the intended use of the created system. For instance, hollow fibres limit multiple healing events (healing several instances of damage in the same area) because of blockage and/or massive release of healing agent. Compartmented fibres allow

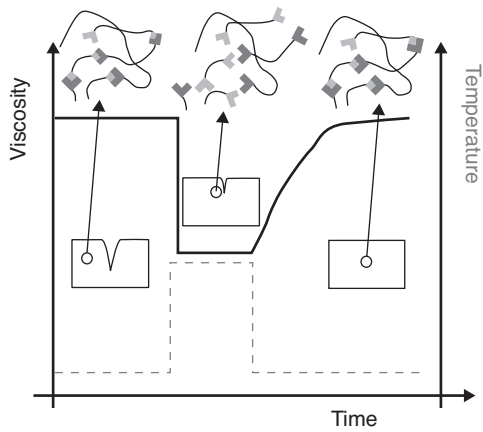


9.4 Existing concepts using embedded fibres: (a) hollow fibre; (b) compartmented fibres and (c) vascular systems.

multiple healing events, each of limited size, due to the amount of healing agent released, so the damage they can heal is smaller than in the other two approaches. On the other hand, vascular systems can also lead to multiple healing events but are very complicated to implement and can lead to blockage under certain conditions.

9.2.2 Intrinsic healing

The so-called intrinsic self-healing polymers are materials capable of repairing the damage via a temporary increase in mobility leading to a re-flow of the material in the damaged area. Such behaviour is based on specific molecular structures and performance of the polymers that enable damage to be healed mostly under specific stimulation (e.g. temperature) followed by a process of restoring the chemical or physical bond strength. Intrinsic self-healing polymers may be seen, therefore, as a 'second generation' self-healing material while being capable of reversibly restoring a damaged material to its original, undamaged state. This healing process is analogous to a zipper which one can open (introducing damage) and then close (initiating repair) (Brochu *et al.*, 2011). Intrinsic healing does not require the addition of a special external healing agent, nor is the healing restricted to single healing events. Although assisted healing is generally required, there are a few concepts that do not require human intervention to heal, as will be discussed in the next section. From the predominant molecular mechanisms involved in the healing processes, intrinsic healing can be achieved through three different modes (MingQiu and MinZhi, 2012): (i) dynamic covalent



9.5 General concept of intrinsic healing. Plot shows temporary drop of viscosity upon local increase of temperature leading to temporary network mobility and damage closure upon removal of stimulus.

chemistry, which comprises the reversible making and breaking of covalent bonds; (ii) thermoreversible physical interactions; or (iii) supramolecular chemistry, which imparts the capability of self-assembly or self-organization using highly directional and reversible non-covalent interactions that dictate the overall mechanical properties of a material. These concepts can in principle be applied to both thermoplastic and thermoset polymers, although until quite recently, intrinsic self-healing of polymers has been restricted to thermoplastic materials. Figure 9.5 shows a schematic of the general principle of healing using physical or covalent bonds reversible intrinsic concepts, where an increase of mobility by, for instance, a temporary loss of network architecture leads to damage closure and restoration.

Wool and O'Connor (1981) systematically studied the theory involved in the healing of polymers. As they pointed out, the healing process goes through five phases: (i) surface rearrangement (ii) surface approaching (iii) wetting (iv) diffusion, and (v) randomization. However, reversible repair of covalently bonded polymers is kinetically inaccessible because the energy barrier to molecular rearrangement is high and the molecular dynamics are slow. Consequently, all second generation self-healing polymer systems currently under investigation concern (i) the chemistry of weak bonds, either of covalent or of non-covalent nature that are reversible at low temperatures or (ii) techniques that input the energy necessary for molecular rearrangement. Regardless of temperature and absolute time scale, however, the two approaches require that local reversibility (i.e. bond forming–breaking reactions) must be significantly faster than global processes (e.g. polymer flow

and macroscopic deformation). The design of intrinsic self-healing polymeric systems may therefore involve hybrid architectures that possess both reversible bonded parts as well as irreversibly bonded parts to overcome shortcomings of an entirely reversible system formed by rather weak bonds. Such a requirement for hybrid architecture is the consequence of introducing increased mobility within the material which leads to generally lower mechanical properties.

Reversible covalent chemistry

A dynamic dissociation and re-association of stress-bearing bonds allow for rapid conformational changes that ensure the healing action as reaction on damage. The success of the healing operation depends on the characteristics of the association, the overall flexibility of the molecule, and the environment of the system. Such chemistry has a long history in polymer science ranging from ring-chain equilibria and reactive polymer blends to controlled (living) free radical polymerization allowing a reorganization of the material architecture. Current dynamic covalent systems comprise a wide range of well-known reaction types including ring-chain equilibria and chain-exchange reaction as in the case of cross-linked poly(dimethylsiloxane) (Zheng and McCarty, 2012), retro Diels-Alder (DA) reactions (Chen *et al.*, 2002; Chujo *et al.*, 1990; Craven *et al.*, 1969; Murphy *et al.*, 2008), disulfide (Canadell *et al.*, 2011; Otsuka *et al.*, 2010) and trithiocarbonate exchange reactions (Amamoto *et al.*, 2011), reversible hydrazone linkages (Deng *et al.*, 2010) and alkoxyamines (Otsuka *et al.*, 2007). These, as well as diarylbenzofuranone (Imato *et al.*, 2012), mostly depend on a trigger/stimulus such as thermal energy, but other triggers for healing can also be found in the literature such as irradiation for opening/closing a 4 + 4 photo-cycle (Froimowicz *et al.*, 2011; Ghosh and Urban, 2009), pH changes (Deng *et al.*, 2010) or even catalytic additives in order to perform their healing action.

Reversible physical interactions

Ionomers are possibly the most significant kind of polymers using physical interactions for self-healing materials. These are systems containing species like acid groups in the form of metal salts (ionic species) bonded to the structure of the organic polymer creating electrostatic interactions or aggregates and therefore having a large effect on the final mechanical and physical properties of the material. The interest in such systems in terms of self-healing relies on the transitions and relaxations that the ionic clusters undergo under certain temperature ranges leading to mobility of the polymeric network and significant change of the mechanical properties (Tachino

et al., 1993). Ionomers have been extensively studied for ballistic healing (Kalista, 2009; Varley and van der Zwaag 2008) and have more recently been studied for applications as protective coatings (Garcia *et al.*, 2011c; Mathis *et al.*, 2010).

Reversible supramolecular interactions

Reversible supramolecular interactions in polymers can be achieved via several mechanisms, such as: hydrogen bonding, exemplified by the ureidopyrimidinone unit (Bosman *et al.*, 2004; Montarnal *et al.*, 2009), metal coordination chemistry (Fiore *et al.*, 2011; Kumpfer *et al.*, 2010), or π-π stacking, such as that seen with triphenylene units (Buratini *et al.*, 2009). These materials can heal autonomously in a few cases, but in most cases also need stimuli for their healing action such as temperature or pressure.

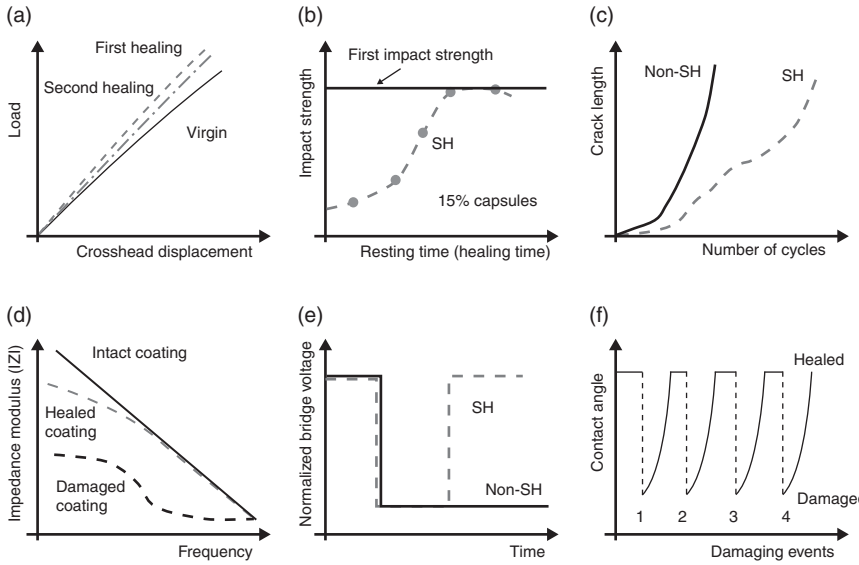
As mentioned previously, the main goal of introducing healing mechanisms in polymeric systems is to give them the ability to restore local loss of functionality so that the material, system or product can be used for a longer time without replacement and with minimum maintenance (Garcia *et al.*, 2011a). For this reason the following section is dedicated to existing examples aimed at healing a certain damaging type by extrinsic and intrinsic approaches. Nevertheless, it should be highlighted that the field is relatively young and that many more applications will appear in the coming years aiming to restore unexplored functionalities.

9.3 Self-healing and recovery of functionality in materials

Figure 9.6 gives an indication of the effect of healing of several functionalities or material properties. In order to show the restoration of properties and functionalities we have chosen to plot how the recovery of each property can be observed based using different lab techniques such as electrochemical impedance spectroscopy (EIS), contact angle measurements or traditional impact tests. Through the rest of the paper, references to this figure will be frequent, explaining the most relevant features to be observed when evaluating self-healing systems which restore certain functionalities.

9.3.1 Recovering structural integrity

The concept of self-healing was first clearly proven using extrinsic healing approaches to restore structural integrity (White *et al.*, 2001) and thus it is in



9.6 Healing functionalities as evaluated by several techniques. (a) toughness recovery (Caruso, 2008); (b) impact strength recovery (Yao, 2011); (c) fatigue resistance recovery (Yuan, 2011); (d) corrosion protection recovery (Garcia, 2011); (e) electrical conductivity recovery (Odom, 2012) and (f) hydrophobicity recovery (Liu, 2012).

this particular topic where more papers can be found using different extrinsic and, more recently, intrinsic approaches.

Fracture toughness recovery

The initial work of White *et al.* (2001) to demonstrate the healing capabilities of encapsulated systems was based on load-displacement tests showing that a healed sample could recover up to 75% of the virgin fracture load. Despite 'load at break' being representative of healing, it is not considered the most appropriate parameter to evaluate healing or healing efficiency due to test inaccuracies related to improper alignment of healed surfaces and surface roughness effects. For these reasons, when using tensile testing, fracture toughness is chosen as the most relevant parameter as it decreases related experimental errors (Fig. 9.6a). In this direction, many communications have been published using different encapsulation approaches such as the dual encapsulation approach, encapsulation–catalyst, and finally, solvent encapsulation (Caruso *et al.*, 2007, 2008; Jin *et al.*, 2012; Mauldin *et al.*, 2007). Kessler *et al* (2003) showed how encapsulated dicyclopentadiene with dispersed Grubbs catalyst partially restored the initial toughness of an epoxy

matrix and how a slight increase of the healing temperature (from room temperature to 80°C) further increased the healing efficiency to almost reach initial toughness values. In a more recent work, Jin *et al*. (2012) showed the effect of amine-capsule content in the fracture toughness recovery of epoxy dual encapsulation systems. Although the virgin toughness does not change with amine-capsule content, the healed toughness changes to reach an optimum, dropping gradually afterwards. Caruso *et al*. (2008) also described in their paper that at least three to five healing events were possible with a solvent encapsulated system. This work gains relevance if we consider that encapsulated systems normally have the disadvantage of showing a single healing event.

Impact strength recovery

Yao *et al.* (2011) have recently proposed a system that efficiently heals upon impact. The system used by the authors was a polystyrene (PS) network synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization with embedded capsules containing glycidyl methacrylate (GMA). Upon impact the capsules break internally releasing the GMA which then infiltrates the cracks. Under certain temperature and time conditions, the GMA copolymerizes with the PS matrix eventually covalently bonding the crack planes so that cracks do not propagate further and the system can partially recover the initial impact strength. The authors showed in their work how, with the capsule content, the initial impact strength gradually decreases while the impact strength after healing increases. An optimum balance between initial properties and residual static strength after impact was found at 15% capsule content (Fig. 9.6b).

Xiao *et al*. (2009) have proposed a self-healing epoxy matrix based on cationic polymerization and evaluated the effect of capsule content in the healing efficiency after impact. The authors showed that the proposed system could reach its maximum healing efficiency at around 20 min of healing time after impact, while no variations in healing efficiency were observed within five months storage prior to impact. Other healing systems using epoxy/mercaptan dual encapsulation have shown stability of 11 months with 100% healing after storage time (Yuan *et al.*, 2011).

Fatigue resistance recovery

The use of encapsulated systems to stop or retard fatigue crack growth using a succession of loading and healing cycles, thus extending fatigue life, has been explored by several groups. One of the first attempts in this area was done by Brown *et al*. (2005) using dicyclopentadiene capsules with Grubbs catalyst dispersed in the matrix where the authors showed that

the concept worked. In this publication it was highlighted that the degree of fatigue life extension was dependent on a balance between the relative magnitude of mechanical kinetics of crack propagation and the kinetics of healing. In other words, healing can occur only if the fatigue loading is periodically stopped to allow partial or full crack healing, an idea extendable to all healing systems where the loading/damaging event has to stop to allow healing.

Yuan *et al.* (2011b) employed another approach, this time using a double encapsulation system with one capsule type filled with an epoxy prepolymer (i.e. diglycidyl ether of bisphenol A, DGEBA) and a second capsule type filled with a mercaptan/tertiary amine as hardener (i.e. pentaerythritol tetrakis (3-mercaptopropionate)). In this work the authors show how encapsulated systems were able to reduce the crack growth by gluing both crack planes with the healing agents, thus extending the number of cycles until failure and thereby extending the service lifetime of the sample (Fig. 9.6c).

Damage recovery in ballistic impact testing

When a system is impacted by a bullet (or other high speed object), normally the bullet leaves a hole behind which allows mass transport (e.g. of a liquid) from one side of the sample to the other. In the case of flammable or toxic liquids or gases such mass transport may result in more damage than the impact itself. This is, therefore, a field where self-healing is potentially very interesting, aiming to close damage after impact. In this field, ionomers (intrinsic approach) are probably one of the most successful systems studied so far. Ionomers have been broadly investigated for thermomechanic healing after ballistic puncture/impact or sawing damage (Kalista, 2009; Varley and van der Zwaag, 2008). The self-healing occurs through a heat generated frictional process, which heats the polymer to the viscoelastic melt state and provides the ability to rebound and repair damage thanks to existing clusters in the matrix. By contrast, low speed friction events fail to produce sufficient thermal energy to initiate healing. The combination of elastic flexibility, high melt strength and spontaneous formation of physical cross-links gives the ionomers a self-healing behaviour upon ballistic impact.

9.3.2 Recovering surface aesthetics, barrier and corrosion protection

The recovery of surface aesthetics, barrier and corrosion protection can be mainly, though not uniquely, linked to the field of coatings science, and thus it is in these fields where most of the approaches and studies have been

performed, often implementing the same concepts of polymer science and sometimes looking for applications for a specific functionality they aim to heal, such as corrosion protection, which requires specific solutions for a really successful approach.

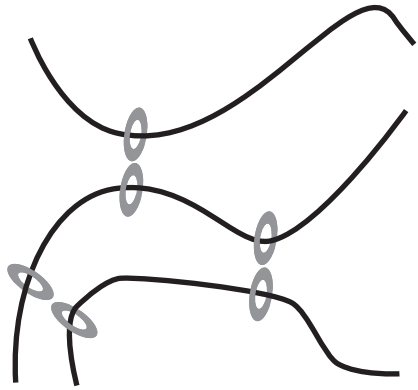
Surface aesthetics recovery

In extrinsic approaches, such as capsules, the scar material used for healing is normally of a nature different from the material it repairs; thus after repair a clear visible scar is left with properties different from those of the bulk material. Intrinsic approaches, on the other hand, use exactly the same material as the surrounding matrix to restore a given functionality or property, thus leading to less-visible scars, if they are visible at all. For this reason, the recovery of surface finish and aesthetics is mainly achieved using intrinsic healing concepts. Polymeric coating systems with low transition temperatures which soften under the influence of solar radiation on a sunny day or the use of other sources directly applied on the surface producing low temperatures are the simplest approach. In this case, the healing is triggered when the environmental temperature is higher than the softening temperature of the polymer or one of its phases ($T_{env} > T_g$ or T_m). Surface tension will ensure a minimization of the surface area and thus the recovery of small superficial damage, although under proper conditions and chemistry the systems could be capable of healing larger areas of damage. So far, this is the only self-healing approach fully commercialized for organic coatings: Bayer and Sikkens-Akzo Nobel developed isocyanate-based varnishes which heal by a concept known as retarded elasticity (Garcia *et al.* 2011a). Recently, Garcia *et al*. (2011c) and Mathis *et al.*, (2010) reported scratch healing behaviour of ionomers in an explorative study to identify the possibility of such approaches for use in self-healing coatings.

The intrinsic self-healing concept as introduced by Nissan is based on a fundamentally different system (Kohzo, 2007), namely on so-called slide-ring-technology: polymer chains which are topologically linked through freely movable cross-links (Fig. 9.7). This concept ensures that the polymer chains can freely equalize the tensions of threaded polymers after mechanical damage, thus potentially leading to deformation induced healing systems.

Barrier and corrosion protection recovery

Both extrinsic and intrinsic approaches have been employed to restore barrier and corrosion protection. Several works can be found in the



9.7 Slide movable cross-links. (*Source*: Adapted from Kohzo, 2007.)

literature using encapsulated agents for restoration of barrier and corrosion protection. Cho *et al.* (2009) used an encapsulated-catalyst system to protect steel from corrosion at scribed areas, showing relevant protection in salt fog spray tests. Kumar *et al.* (2006) and Samadzadeh *et al.* (2011) used the encapsulation of a single reactive healing agent (i.e. tung oil) that reacts with ambient humidity. Despite these systems showing good corrosion protection, several doubts about long-term protection have been posed due to the incomplete coverage of the underlying metallic surface and possible entrapment of corrosive species during the healing process – key issues in corrosion protection (Garcia *et al.*, 2011a). In order to advance solving these issues Garcia *et al*. (2011b; Gonzalez-Garcia *et al*., 2011) proposed a new system which was specifically made for corrosion protection of aluminium substrates but was extendable to other metallic surfaces. The new concept showed excellent long-term protection when measured by EIS, scanning vibrating electrode technique (SVET) and scanning electrochemical microscopy (SECM). The concept proposed is based on silyl esters and presents several advantages with respect to previous ones for corrosion protection: (i) it is based on surface healing instead of volumetric-gap filling; (ii) does not require catalyst or cross-linker as surface hydroxides and environmental humidity suffice for reaction to occur; and (iii) the reaction takes place only in the case of corrosion damage. Figure 9.6d shows an example of the effect of healing on the corrosion protection of a damaged coating using EIS, where the healing is detected by an increase of the total impedance of the system.

The concept of encapsulation to restore barriers has also been employed for applications other than corrosion protection, for instance

in the packaging industry, although not many examples can be found yet. Andersson *et al*. (2009) studied the feasibility of implementing single agent encapsulation on paperboards highlighting the potential of the concept, as the healing agents locally reinforced the system leading to a decrease in fibre damage.

For the repair of larger damage than superficial scratches of a coating, approaches using mobility based on T_g are not sufficient. The use of more complex chemistries such as dynamic covalent bonds and supramolecular chemistry is then necessary. The retro-DA reaction is particularly useful for that, especially if the active components are used in powder coating systems (Wouters *et al.*, 2009, 2011), where reversibility was found at temperatures around 150°C. Also the aforementioned disulfide based systems (Canadell *et al.*, 2011; Yoon *et al.*, 2012) as well the photo-induced, reversible cross-linking of antracene containing networks (Froimowicz *et al.*, 2011) show repeated healing possibilities for surface damage. Supramolecular approaches may be also applied in the restoration of damaged coatings as proposed by Bosman *et al*. (2004). Although the previous systems have mostly been evaluated in terms of crack or scratch closure in free standing films and the quality of the scar formed has not been addressed or quantified in terms of sealing level, some studies have shown the potential of intrinsic approaches for corrosion protection using shape memory polymers (Jorcin *et al.*, 2011).

9.3.3 Recovering interfacial bond strength between dissimilar materials

Adhesion between similar materials (e.g. polymer A–polymer A) as well as between dissimilar materials (e.g. metal–polymer, polymer A–polymer B) is a critical aspect in many fields such as coatings technology and composite materials. As highlighted in one of our previous works (Garcia *et al.*, 2011a) loss of adhesion is a critical factor in coatings technology leading to accelerated localized corrosion. In other fields of application, including packaging and electronic devices such as light emitting diodes (LEDs), loss of adhesion is one of the most critical reasons for device malfunction (Lafont *et al.*, 2012). It is clear that many application fields could significantly benefit from the implementation of self-healing concepts aiming at interfacial bonding restoration. So far few studies have been published on this topic although research is on in different research centres. Jin *et al*. (2011) recently published a paper addressing the topic with encapsulated dicyclopentadiene (DCPD) and dispersed Grubbs catalyst in an adhesive layer. Their results, despite showing promise, have not yet shown full adhesion recovery because the

studied adhesive showed a high component of cohesive failure, thus making it difficult to discern the restoration at the interface of the dissimilar metals (adhesive healing).

Intrinsic healing systems based on supramolecular chemistry also have their value for interfacial bonding restoration despite most of the supramolecular material displaying only moderate mechanical properties. The ureidopyrimidinone unit based materials as described by van Gemert *et al*. (2012) are not only able to show impressive abilities of recovery for surfaces of coatings but can also be considered for use in mendable adhesive applications. Moreover, it should be noted that almost all approaches related to healing functionalities of composites discussed later, can be considered as systems healing the interfaces between dissimilar materials.

9.3.4 Recovering electrical conductivity

Not many works have been yet published in the field of restoring electrical (or thermal) conductivity. So far the most widely explored approach to restoring electrical conductivity is based on encapsulation of electrically conductive elements in or as a liquid medium. Caruso *et al*. (2009) proposed the encapsulation of carbon nanotubes (CNTs) suspended in non-polar solvents such as chlorobenzene (PhCl) and ethyl phenylacetate (EPA). Odom *et al*. (2010) encapsulated solutions of conductive tetrathiafulvalene–tetracyanoquinodimethane (TTF-TCNQ) and Blaiszik *et al*. (2012) encapsulated eutectic Ga-In alloy (which melts above 16°C).

Another approach is the use of encapsulated solvents included in soluble conductive polymers. Capsules will break upon damage, dissolve the matrix and restore the electrical contact between phases once the solvent has evaporated (Odom *et al*., 2012) as can be observed by the electrical conductivity test in Fig. 9.6e in which a drop of potential is detected when the system is damaged and restored after a period of time when a self-healing system is employed. The restoration of electrical (and thermal) conductivities is expected to significantly grow in the near future due to the broad application in many different fields.

9.3.5 Recovering hydrophobicity and hydrophilicity

(Super)hydrophobic or (super)hydrophilic surfaces have attracted the interest of many research groups and companies in recent years but despite the many concepts already developed, the main challenge for a successful final application is the durability of the surfaces designed. In this respect, the implementation of self-repair can potentially help their final

implementation. For these reasons several studies have been published using both intrinsic and extrinsic healing approaches of hydrophobic and hydrophilic surfaces.

In this direction a special but very interesting and original example of healing is the use of polymer networks with dangling fluorinated side groups which are able to restore hydrophobicity (Dikić, 2006, 2012). Here the normally observed surface segregation of fluorinated parts of the system and the subsequent loss of the hydrophobicity after surface damage is turned to a self-replenishing effect by homogenously covalently bonding the fluorinated units within the network of the polymer. Upon damage, the mobile side groups (i.e. dangling chains) in close proximity to the surface will restore the hydrophobicity of the system while moving to the outer surface due to their low surface energy.

Extrinsic approaches have also been used to restore hydrophobicity. For instance, Wang *et al*. (2011) have recently proposed an approach inspired by nature. This system, despite not being polymeric in nature plays with concepts that can be relatively easily transferred to polymeric ones. It consists of nanoporous alumina as substrate in which the nanopores are filled with an oleophobic liquid (perfluorooctyl acid). In this way, a typical oleo- and hydrophilic surface became hydrophobic and oleophobic. The healing capacity of the system was demonstrated by O_2 plasma etching. A determination of the contact angle shows that the system is constantly becoming hydrophobic by the continuous supply of the contained liquid driven by minimization of surface tension in the structure. Repeated healing was also demonstrated, the only limitation being the amount of hydrophobic liquid contained in the nanostructure.

In the same direction as the previously described concept, Wong *et al.* (2011) proposed another system and named it as slippery liquid-infused porous surface(s) (SLIPS). In this case, nonetheless, the system is purely polymeric. The matrices used a periodically ordered epoxy resin-based nanostructure and a random network of Teflon nanofibrous membranes, where perfluorinated liquids were infused. The researchers showed that the surfaces can keep the hydro- and oleophobicity even after the surface has been severely mechanically damaged.

Liu *et al*. (2012) have recently proposed a sol–gel coating formed by silica containers doped with octadecylamine (ODA) and coated with dopamine-ODA, while tetraethoxysilane was used as cross-linker for the coating network. The developed coating has shown that, after removal of the surface hydrophobic layer (ODA) with O_2 plasma, the system becomes first hydrophilic (due to the suppression of the surface hydrophobic layer) but with time and high relative environmental humidity the coating again becomes superhydrophobic due to release of encapsulated ODA, as measured by contact angle measurements (shown in

Fig. 9.6f). Moreover, the system thus developed showed repeated healing (up to 25 times).

Another intrinsic approach has been proposed by Li *et al.* (2010). This time, the polymer network itself was used to heal the hydrophobicity by molecule displacement. The coating employed was made using layer-by-layer deposition of poly(allylamine hydrochloride) (PAH) with sulfonated polyether-ether-ketone (SPEEK) and polyacrylic acid (PAA) with final addition of 1H,1H,2H,2H-perfluorooctyltriethoxysilane (POTS) by chemical vapour deposition (CVD) on the PAH-SPEEK/PAA coating. After removal of surface layers by O_2 plasma, hydrophobicity was restored as a function of time and relative ambient humidity.

9.3.6 Recovering mechanical properties of fibre composites

The implementation of self-healing concepts in fibre composites has received quite significant attention since the early beginnings of the field and is thus treated here as a separate sub-section. Several applications within fibre composites have been explored so far, such as sandwich structures, glass and carbon fibre reinforced composites, and woven composites. Fibre composites can fail both in compression and tension, leading to: (i) loss of adhesion between fibre and matrix (i.e. delamination); (ii) cracking of the polymeric matrix; and (iii) fibre breakage. The most common of these failures is delamination which has thus received most attention. It is believed that the implementation of interfacial healing in fibre–matrix composites before damage propagates will lead to higher damage tolerance and reliability of structures made thereof.

In order to evaluate whether the self-healing concept works in fibre composites, several techniques have been used including impact, four point bending, low speed impact and indentation, while to detect the healing extent the same techniques can be used. Other non-destructive techniques are gaining more and more interest as useful tools to follow the extent of damage and healing such as X-ray computed tomography (McCombe *et al.*, 2012; Mookhoek *et al.*, 2010).

In 2010, Blaiszik *et al.* (2010b) combined the use of fibres with spherical capsules to restore the adhesion between fibre and matrix. In their approach they coated E-glass fibres with Grubbs catalyst and with 1.5 μm diameter capsules containing dicyclopentadiene. This approach did not affect the intrinsic mechanical properties of the fibres and the composite while the system showed significant, although not optimal, interfacial shear strength recovery.

Recently, Hamilton *et al*. (2012) showed how vascular systems using pumped healing agents through microcapillaries can deliver locally and, when needed, large amounts of healing agent. Moreover, the system has shown that several healing events are possible (up to 15 times) clearly outperforming static-unpressurized vascular systems which rely on capillary forces. The concept nevertheless has a chief disadvantage: the complexity of fabrication limits its applicability.

Hollow fibres have also been used not only to heal damage after indentation (impact) but to detect damage using ultraviolet mapping of released dye-containing liquid (Pang and Bond, 2005). The introduction of hollow fibres in fibre composites can have a negative effect in the composite mechanical properties. In this direction, Kousourakis and co-workers (Kousourakis and Mouritz, 2010) reported the effect of hollow fibres in both compression and tension properties of composites with longitudinal and transverse fibres. The authors concluded that when located longitudinal to the applied stress, hollow fibres have almost no effect on elastic modulus or on failure strength. When the fibres are oriented transversal, a critical diameter of fibres of 200 μm was detected after which the initial mechanical properties were significantly affected. Finally, it was concluded that hollow fibres can increase the damage tolerance of composite materials and *T*-joints. More recently Norris *et al* (2011a, 2011b) evaluated the effect of different hollow fibre-composite fabrication methods, including fibre placement in the composite, on the mechanical properties, showing the importance of the fibre-composite geometry and hollow fibre placement with respect to damaging properties such as fracture toughness and impact delamination.

Besides extrinsic healing concepts for fibre composites, intrinsic concepts in particular have already been explored. First attempts using thermoplastic/thermosetting semi-interpenetrating networks as a material displaying repeatable self-healing ability. Jones and co-workers introduced a soluble linear polymer to a thermosetting epoxy resin (Hayes *et al*., 2007a, 2007b). The chosen thermoplastic was poly(bisphenol-A-*co*-epichlorohydrin), which is highly compatible with the DGEBA resin matrix. Upon heating a damaged resin system, the thermoplastic material would mobilize and diffuse through the thermosetting matrix facilitating healing. When this healable resin was compounded with cross-ply glass fibre, effective healing of transverse cracks and delamination in the composites was demonstrated. Alternatively, thermosets containing polyethylene-co-methacrylic acid (EMAA) particles or fibres act in the same way in a fibre composite (Meure *et al.*, 2010), whereas in this case, upon heating, the liquefied EMAA rebonds damaged areas with a healing extent of 100%. The healing in this case is due to melting but also to the side reaction of the liquid

EMAA acidic groups with residual oxirane and hydroxyl units of the matrix ensuring a good adhesion between EMAA and the epoxy resin matrix. Interestingly, in this particular case of a temperature triggered healing system, a higher toughness composite was frequently obtained as compared to the initial composite before damage due to bridging EMAA material after the healing event (Pingkarawat *et al.*, 2012; Varley and Parn, 2012; Wang *et al.*, 2012; Yang *et al.*, 2012).

Dynamic covalent chemistry has also been applied to recover the functionalities of fibre composites. In this case one of the most suited material systems is certainly a polymeric material with incorporated DA linkages. Upon heating, a thermoset cross-linked using DA moieties will transfer in a low viscosity liquid allowing not only the necessary mobility to flow and heal the damaged polymeric matrix but also the ability to wet the delaminated fibres and to embed/bond them again within the composite and glue the fractured fibre surfaces together. A further improvement of this concept can be achieved while also using the DA strategy to rebond the fibres via functionalization of the fibre surfaces with DA groups, as shown by Peterson *et al*. (2011). These systems show a high recovery of the intrinsic properties (up to 100%) in up to five consecutive healing cycles.

9.4 Conclusion

Materials and systems which are able to react to damage and repair it have been a dream of constructors and designers for a long time. The first explorative experiments and developments using extrinsic healing approaches such as fibres and spherical capsules established the principle and basis for the implementation of the self-healing concept into materials and systems. However, the biggest drawback of implementing extrinsic healing approaches has been, for almost all cases, the partial deterioration of the original properties (e.g. mechanical resistance decrease) to an extent which made the use of self-repair non-economical. This drawback was later extrapolated to intrinsic concepts. Fortunately, work in the field has focussed in recent years not only on the development of new and different strategies and principles of self-repair but also on the implementation of self-repair of damaged functions of systems in practice. The combination of both lines of development (i.e. new concepts and applications) has resulted in a number of new as well as further developed applications of self-repair which continuously grows.

In this paper we proposed a large tool-box of possible useable mechanisms and the associated chemistry/physics of repair, consequently increasing the possibilities of incorporating self-healing functionalities without drastically decreasing other properties. Moreover, several applications of self-healing

concepts have been proposed based on the type and size of damage to be repaired. It is foreseeable that, in time, self-repair in functional materials such as electrical components used in critical areas like space exploration and light emitting diodes will become a standard option just like current electronic chips and devices contain self-healing functionalities and algorithms to reduce electronic failures.

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9.6 References

Amamoto Y., Kamada J., Otsuka H., Takahara A. and Matyjaszewski K. (2011), Repeatable photoinduced self-healing of covalently cross-linked polymers through reshuffling of trithiocarbonate units, *Angew. Chem. Int. Ed.*, **50**, 1660–1663.

Andersson C., Järnström L., Fogden A., Mira I., Voit W., Zywicki S. and Bartkowiak A. (2009), Preparation and incorporation of microcapsules in functional coatings for self-healing of packaging board, *Packaging Technol. Sci.*, (**22**) 75–291.

Blaiszik B.J., Baginska M., White S.R. and Sottos N.R. (2010b), Autonomic recovery of fiber/matrix interfacial bond strength in a model composite, *Adv. Funct. Mater*., **20**, 3547–3554.

Blaiszik B.J., Kramer S.L.B., Grady M.E., McIlroy D.A., Moore J.S., Sottos N.R. and White S.R. (2012), Autonomic restoration of electrical conductivity, *Adv. Mater.*, **24**, 398–401.

Blaiszik B.J., Kramer S.L.B., Olugebefola S.C., Moore J.S., Sottos N.R. and White S.R. (2010a), Self-healing polymers and composites, *Annu. Rev. Mater. Res*., **40**, 179–211.

Bosman A.W., Sijbesma R.P. and Meijer E.W. (2004), Supramolecular polymers at work, *Mater. Today* **7**, 34.

Brochu A.B.W, Craig S. L. and Reichert W.M. (2011), Self-healing biomaterials, *J. Biomed. Mat. Res. A,* **96**, 492.

Brown E.N., White S.R. and Sottos N.R. (2005), Retardation and repair of fatigue cracks in a microcapsule toughened epoxy composite – Part II: In situ self-healing, *Compos. Sci. Technol.*, **65**, 2474–2480.

Burattini S., Colquhoun H.M., Greenland B.W. and Hayes W. (2009), A novel self-healing supramolecular polymer system, *Faraday Discuss*, **143**, 251–264.

Canadell J., Goossens H. and B. Klumperman (2011), Self-healing materials based on disulfide links, *Macromolecules*, **44**, 2536–2541.

Caruso M.M., Blaiszik B.J., White S.R., Sottos N.R. and Moore J.S. (2008), Full recovery of fracture toughness using a nontoxic solvent-based self-healing system, *Adv. Funct. Mater*., **18**, 1898–1904.

Caruso M.M., Delafuente D.A., Ho V., Sottos N.R., Moore J.S. and White S.R. (2007), Solvent promoted self-healing epoxy materials, *Macromolecules*, **40**, 8830–8832.

Caruso M.M., Schelkopf S.R., Jackson A.C., Landry A.M., Braun P.V. and Moore J.S. (2009), Microcapsules containing suspensions of carbon nanotubes, *J. Mater. Chem.*, **19**, 6093–6096.

Chen X., Dam M.A., Ono K., Mal A., Shen H., Nutt S.R., Sheran K. and Wudl F. (2002), A thermally re-mendable cross-linked polymeric material, *Science*, **295**, 1698.

Cho S.H. Cho, S.R. White and P.V. Braun (2009), Self-healing polymer coatings, *Adv. Mater*. **21**, 645.

Chujo Y., Sada K. and Saegusa T. (1990), Reversible gelation of polyoxazoline by means of Diels-Alder reaction. *Macromolecules*, **23**, 2636–264.

Craven J.M. (1969), US patent 3.435.003.

Deng G., Tang C., Li F., Jiang H. and Chen Y. (2010), Covalent cross-linked polymer gels with reversible sol-gel transition and self-healing properties. *Macromolecules*, **43**, 1191–1194.

Dikić T. (2006), Self-replenishing low-adherence polymer coatings, PhD thesis, Eindhoven University of Technology.

Dikić, T., Ming, W., Van Benthem, R.A.T.M., Esteves, A.C.C. and De With, G. (2012), Self-replenishing surfaces, *Adv. Mater.*, **24**(27), pp. 3701–3704.

Dry C.M. and Sottos N., in: K. Varadian (Ed.) (1993), Smart Structures and Materials 1995: Smart Materials, Proc. SPIE vol. **1916** 438–444.

Fiore G.L., Rowan S.J. and Weder C. (2011), Light-activated healing of metallosupramolecular polymers, *Chimia (Aarau)*, **65**(9), 745.

Fischer H.R. (2010), Self-repairing material systems–a dream or a reality?, *Nat. Sci.,* **2**, 873–901.

Froimowicz P., Frey H. and Landfester K. (2011), Towards the generation of self-healing materials by means of a reversible photo-induced approach, *Macromol. Rapid Commun.*, **32**, 468–473.

Garcia S.J., Fischer H.R. and van der Zwaag S. (2011a), A critical appraisal of the potential of self healing polymeric coatings, *Prog. Org. Coat*., **72**, 211–221.

Garcia S.J., Fischer H.R., White P.A., Mardel J., González-Garcia Y., Mol J.M.C. and Hughes A.E. (2011b), Self-healing anticorrosive organic coating based on an encapsulated water reactive silyl ester: Synthesis and proof of concept, *Prog. Org. Coat*., **70**, 142–149.

Garcia S.J., Varley R.J. and van der Zwaag S. (2011c), A quantitative description of the surface healing of ionomers, Proceedings 3rd International Conference on Self-Healing Materials, Bath, UK.

Ghosh B. and Urban M.W. (2009), Urban MW. Self-repairing oxetane-substituted chitosan polyurethane networks, *Science*, **323**(5920), 1458–460.

Gonzalez-Garcia, Y., Garcia, S.J., Hughes, A.E. and Mol, J.M.C. (2011), A combined redox-competition and negative-feedback SECM study of self-healing anticorrosive coatings, *Electrochem. Comm.*, **13**, 1094–1097.

Gragert M., Schunack M. and Binder W.H. (2011), Azide/alkyne-'click'-reactions of encapsulated reagents: Toward self-healing materials, *Macromol. Rapid Commun*., **32**, 419–425.

Hamilton A.R., Sottos N.R. and White S.R. (2012), Pressurized vascular systems for self-healing materials, *J. R. Soc. Interface*, **9**, 1020–1028.

Hayes S.A., Jones F.R., Marshiya K. and Zhang W. (2007a), A self-healing thermosetting composite material, *Compos Part A-Appl S*, **38**, 1116–1120.

Hayes S.A., Zhang W., Branthwaite M. and Jones F.R. (2007b), Self-healing of damage in fibre-reinforced polymer-matrix composites, *J Roy Soc. Interface*, **4**, 381–387.

Imato K., Nishihara M., Kanehara T., Amamoto Y., Takahara A. and Otsuka H. (2012), Self-healing of chemical gels cross-linked by diarylbibenzofuranone-based trigger-free dynamic covalent bonds at room temperature, *Angew. Chem. Int. Ed*., **51**, 1138–1142.

Jin H., Miller G.N., Sottos N.R. and White S.R. (2011), Fracture and fatigue response of a self-healing epoxy adhesive, *Polymer*, **52**, 1628–1634.

Jin H., Mangun C.L., Stradley D.S., Jeffrey S.M., Nancy R.S. and White S.R. (2012), Self-healing thermoset using encapsulated epoxy-amine healing chemistry, *Polymer*, **53**, 581–587.

Jorcin, J.B., Scheltjens, G., Van Ingelgem, Y., Tourwé, E., Van Assche, G., De Graeve, I., Van Mele, B., Terryn, H. and Hubin, A. (2011), Investigation of the self-healing properties of shape memory polyurethane coatings with the 'odd random phase multisine' electrochemical impedance spectroscopy, *Electrochim. Acta*, **55**, 6195–6203.

Kalista S.J. (2009), Self-healing ionomers. In: Ghosh SK. Ed. *Self-healing Materials: Fundamentals, Design Strategies, and Applications*. Weinheim: Wiley-VCH, 73–100.

Kessler M.R., Sottos N.R. and White S.R. (2003), Self-healing structural composite materials, *Compos.: Part A*, **34**, 743–753.

Kohzo I. (2007), 'Novel cross-linking concept of polymer network: Synthesis, structure, and properties of slide-ring gels with freely movable junctions', *Polym. J.*, **39**, 488–500.

Kousourakis A. and Mouritz A.P. (2010), The effect of self-healing hollow fibres on the mechanical properties of polymer composites, *Smart Mater. Struct*., **19**, 085021.

Kumar A., Stephenson L.D. and Murray J.N. (2006), Self-healing coatings for steel, *Prog. Org. Coat*., **55**, 244–253.

Kumpfer J.R., Jin J. and Rowan S.J. (2010), Stimuli-responsive europium-containing metallo-supramolecular polymers, *J. Mater. Chem.,* **20**, 145–151.

Lafont U., van Zeijl H. and van der Zwaag, S. (2012), Increasing the reliability of solid state lighting systems via self-healing approaches: A review, *Microelectron. Reliab.*, **52**, 71–89.

Li Y., Li L. and Sun J. (2010), Bioinspired self-healing superhydrophobic coatings, *Angew. Chem Int. Ed*., **49**, 6129.

Liu H.A., Gnade B.E. and Balkus K.J. (2008), A delivery system for self-healing inorganic films, *Adv. Funct. Mater*., **18**, 3620–3629.

Liu. Q., Wang X., Yu B., Zhou F. and Xue Q. (2012), Self-healing surface hydrophobicity by consecutive release of hydrophobic molecules from mesoporous silica, *Langmuir*, **28**, 5845–5849.

Mathis C., Garcia S.J. and van der Zwaag S. (2010), Behind the surface healing mechanics of ionized polymers, *Delft Center for Materials Newsletter*, October 5–6.

Mauldin T.C., Rule J.D., Sottos N.R., White S.R. and Moore J.S. (2007), Self-healing kinetics and the stereoisomers of dicyclopentadiene, *J. R.Soc. Interface*, **4**, 389.

McCombe G.P., Rouse J., Trask R.S., Withers P.J. and Bond I.P. (2012), X-ray damage characterisation in self-healing fibre reinforced polymers, *Compos.: Part A*, **43**, 613–620.

Meure S., Furman S. and Khor S. (2010), Poly[ethylene-co-(methacrylic acid)] healing agents for mendable carbon fiber laminates. *Macromol. Mater. Eng.*, **295**(5), 420–4.

MingQiu Z. and MinZhi R. (2012), Design and synthesis of self-healing polymers, *Sci.China Chem.*, **55**, 648.

Montarnal D., Tournilhac F., Hidalgo M., Couturier J.L. and Leibler L. (2009), Versatile one-pot synthesis of supramolecular plastics and self-healing rubbers, *J. Am. Chem. Soc*., **17**(131), 7966–7967.

Mookhoek S.D., Fischer H.R.and van der Zwaag S. (2009), A numerical study into the effects of elongated capsules on the healing efficiency of liquid-based systems, *Comp. Mat. Sci*., **47**(2), 506–511.

Mookhoek S.D. (2010a), Novel routes to liquid-based self-healing polymer systems, PhD thesis, Delft University of Technology, The Netherlands.

Mookhoek S.D., Mayo S.C., Hughes A.E., Furman S.A., Fischer H.R. and van der Zwaag, S. (2010b), Applying SEM-based x-ray microtomography to observe self-healing in solvent encapsulated thermoplastic materials, *Adv. Eng. Mater.*, **12**, 228–234.

Mookhoek S.D., Fischer H.R. and van der Zwaag S. (2012), Alginate fibres containing discrete liquid filled vacuoles for controlled delivery of healing agents in fibre reinforced composites, *Compos, Part A: Appl. Sci. Manuf.*, online August 2012, http://dx.doi.org/10.1016/j.compositesa.2012.08.006.

Murphy, E.B., Bolanos, E., Schaffner-Hamann, C., Wudl, F., Nutt, S.R. and Auad, M.L. (2008), Synthesis and characterization of a single-component thermally remendable polymer network: Staudinger and stille revisited, *Macromolecules*, **41**, 5203–5209.

Nesterova, T.tyana, Dam-Johansen, K. and Kiil, S. (2011), Synthesis of durable microcapsules for self-healing anticorrosive coatings: A comparison of selected methods, *Prog. Org. Coat*., **70**, 342–352.

Nesterova T. (2012), Self-healing anticorrosive coatings, PhD thesis, Technical University of Denmark, Denmark.

Norris C.J., Bond I.P. and Trask R.S. (2011a), The role of embedded bioinspired vasculature on damage formation in self-healing carbon fibre reinforced composites, *Composites: Part A,* **42**, 639–648.

Norris C.J., Bond I.P. and Trask R.S. (2011b), Interactions between propagating cracks and bioinspired self-healing vascules embedded in glass fibre reinforced composites, *Compos. Sci. Technol.,* **71**, 847–853.

Odom S.A. Odom, M.M. Caruso, A.D. Finke, A.M. Prokup, J.A. Ritchey, J.H. Leonard, S.R. White, N.R. and Moore S.J.S. (2010), Restoration of conductivity with TTF-TCNQ charge-transfer salts, *Adv. Funct. Mater.*, **20**, 1721–1727.

Odom S.A., Chayanupatkul S., Blaiszik B.J., Zhao O., Jackson A.C., Braun P.V., Sottos N.R., White S.R. and Moore J.S. (2012), A self healing conducting ink, *Adv. Mater.*, **24**, 2578–2581.

Otsuka H., Aotani K., Higaki Y., Amamoto Y. and Takahara A. (2007), Thermal reorganization and molecular weight control of dynamic covalent polymers containing alkoxyamines in their main chains, *Macromolecules*, **40**, 1429–1434.

Otsuka H., Nagano S., Kobashi Y., Maeda T. and Takahara A. (2010), A dynamic covalent polymer driven by disulfide metathesis under photoirradiation, *Chem. Commun.*, 1150–1152.

Pang J.W.C. and Bond I.P. (2005), A hollow fibre reinforced polymer composite encompassing self-healing and enhanced damage visibility, *Compos. Sci. Technol.,* **65**, 1791–1799.

Pingkarawat K., Wang C.H., Varley R.J. and Mouritz A.P. (2012), *Compos.: Part A,* **43**, 1301–1307.

Peterson A.M., Jensen R.E. and Palmese G.R. (2011), Thermoreversible and remendable glass-polymer interface for fiber-reinforced composites, *Comp. Sci. Technol.*, **71**, 586–592.

Samadzadeh M., Boura S.H., Peikari M., Kasiriha S.M. and Ashrafi A. (2010), A review on self-healing coatings based on micro/nanocapsules, *Prog. Org. Coat.*, **68**, 159–164.

Samadzadeh M., Boura S.H., Peikari M., Ashrafi A. and Kasiriha S.M. (2011), Tung oil: An autonomous repairing agent for self-healing epoxy coatings, *Prog. Org. Coat.*, **70**, 383–387.

Tachino, H. Hara, E. Hirasawa, K. Tadano and S. Yano (1993), Dynamic mechanical relaxations of ethylene ionomers, *Macromolecules* **26**, 752–757.

Toohey K.S., Sottos N.R. and White S.R. (2009), Characterization of microvascular-based self-healing coatings, *Exp. Mech.*, **49**, 707–717.

Van der Zwaag (2007) Springer Series in Materials Science, Vol. 100: *Self-Healing Materials: An Alternative Approach to 20 Centuries of Materials Science* (Ed. S. van der Zwaag), Springer, Dordrecht, The Netherlands.

van Gemert, G.M.L., Peeters, J.W., Söntjens, S.H.M., Janssen, H.M. and Bosman, A.W. (2012), Self-healing supramolecular polymers in action, *Macromol. Chem. Phys.*, **213**, 234–242.

Varley R.J. and Parn G.P. (2012), Thermally activated healing in a mendable resin using a non woven EMAA fabric, *Compos. Sci. Technol.*, **72**, 453–460.

Varley R.J. and van der Zwaag S. (2008), Towards an understanding of thermally activated self-healing of an ionomer system during ballistic penetration, *Acta Mater.*, **56**, 5737–5750.

Wang C.H., Sidhu K., Yang T., Zhang J. and Shanks R. (2012), Interlayer self-healing and toughening of carbon fibre/epoxy composites using copolymer films, *Composites: Part A*, **43**, 512–518.

Wang X., Liu X., Zhou F. and Liu W. (2011), Self-healing superamphiphobicity, *Chem. Commun.*, **47**, 2324–2326.

White S.R. White, N.R. Sottos, P.H. Geubelle, J.S. Moore, M.R. Kessler, S.R. Sriram, E.N. Brown and Viswanathan S. (2001), Autonomic healing of polymer composites, *Nature*, **409**, 794–797.

Williams K.A. Williams, D.R. Dreyer and Bielawski C.W. (2008), The Underlying Chemistry of Self-Healing Materials, *MRS Bulletin*, **33**, 759–765.

Wool R.P. and O'Connor K.M. (1981), A theory of crack healing in polymers, *J. Appl. Phys.*, **52**, 5933.

Wong T.-S., Kang S.H., Tang S.K.Y., Smythe E.J., Hatton B.D., Grinthal A. and Aizenberg J. (2011), Bioinspired self-repairing slippery surfaces with pressure-stable omniphobicity, *Nature*, **477**, 443–447.

Wouters M., Craenmehr E., Tempelaars K., Fischer H., Stroeks N. and van Zanten J. (2009), Preparation and properties of a novel remendable coating concept, *Prog. Org. Coat.,* **64**, 156–162.

Wouters, M., Burghoorn, M., Ingenhut, B., Timmer, K., Rentrop, C., Bots, T., Oosterhuis, G. and Fischer, H. (2011), Tuneable adhesion through novel binder technologies, *Prog.Org. Coat.*, **72**, 152–158.

Xiao D.S., Yuan Y.C., Rong M.Z. and Zhang M.Q. (2009), Self-healing epoxy based on cationic chain polymerization, *Polymer*, **50**, 2967–2975.

Yang T., Wang C.H., Zhang J., He S. and Mouritz A.P. (2012), Toughening and self-healing of epoxy matrix laminates using mendable polymer stitching, *Composites Science and Technology*, **72**, 1396–1401.

Yao L., Yuan Y.C., Rong M.Z. and Zhang M.Q. (2011), Self-healing linear polymers based on RAFT polymerization, *Polymer*, **52**, 3137–3145.

Yoon, J.A., Kamada, J., Koynov, K., Mohin, J., Nicolaÿ, R., Zhang, Y., Balazs, A.C., Kowalewski, T. and Matyjaszewski, K. (2012), Self-healing polymer films based on thiol-disulfide exchange reactions and self-healing kinetics measured using atomic force microscopy, *Macromolecules*, **45**(1), 142–149.

Yan Chao Yuan, Xiao Ji Ye, Min Zhi Rong, Ming Qiu Zhang, Gui Cheng Yang and Jian Qing Zhao (2011), Self-healing epoxy composite with heat-resistant healant, *ACS Appl. Mater. Interfaces*, **3**, 4487–4495.

Yuan Y.C., Rong M.Z., Zhang M.Q., Yang G.C. and Zhao J.Q. (2011), 'Self-healing of fatigue crack in epoxy materials with epoxy/mercaptan system'*, eXPRESS Polym. Lett.,* **5**(1), 47–59.

Zheng P. and McCarty T.J. (2012), A surprise from 1954: Siloxane equilibration is a simple, robust, and obvious polymer self-healing mechanism, *J. Am. Chem Soc*., **134**, 2024–2027.

Part II

Applications of smart polymers

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10

Smart instructive polymer substrates for tissue engineering

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Abstract: The development of instructive substrates with applications in tissue regeneration has become the focus of intensive research in the recent years. The term 'instructive' is applied to responsive polymeric substrates that are able to regulate cell behavior in response to external factors. This chapter will describe reported strategies to combine material responses to environmental changes with signaling molecules for generating well-controlled cellular microenvironments. Biomaterials that respond to temperature, pH, light, electrical or magnetic fields, or to the presence of biochemical signals are highlighted, together with their application in tissue engineering strategies.

Key words: tissue engineering, bioinstructive systems, engineered biomaterials, customized microenvironments.

10.1 Introduction

Tissue engineering (TE) emerged with the aim of creating substitutes that replace or restore a damaged tissue or organ and significantly improve the quality of life of millions of patients (Vacanti, 2006). Typical strategies in TE combine engineered materials (scaffolds), patient cells, and signaling molecules. Most of the TE techniques currently in use rely on the use of passive scaffolds that support initial cell attachment and subsequent tissue regeneration (Nair and Laurencin, 2007; Ratner and Bryant, 2004). The emerging trend in this field is the creation of instructive scaffolds that allow precise manipulation of cellular processes by external control of cell–material interactions (Kim and Hayward, 2012; Nelson and Tien, 2006). Given the high complexity of most of the tissues in the human body it is essential to provide specific physical and biochemical signals in order to regulate cell behavior and promote tissue regeneration. Spatiotemporal control over the distribution of biological cues within a biomaterial is of particular interest in

the development of functional tissues, but the mechanical properties of the scaffold and their change over time are also relevant issues. The overall challenge is to create biomaterials with dynamic and tunable properties mimicking the active microenvironment that occurs *in vivo*. This is a concept that differs considerably from traditional biomaterials approaches, where the properties of the material were most likely not tunable in space or time. Most of the recent reported work in this area uses temperature, pH, electric field or light to tune the bulk properties of a biomaterial (Delair, 2012; Mano, 2008). New systems that allow spatial control of bioactive cues to induce specific cellular responses within three-dimensional (3D) structures are emerging and driving the development of smart 3D structures for TE strategies.

This chapter gives an overview on the use of instructive systems in TE strategies, including the description of the chemical systems used and their advanced applications in different areas of TE. Future trends in the field will be also discussed at the end.

10.2 Instructive polymeric surfaces

The importance of cell–surface interactions is a key step for the successful application of any biomaterial. Therefore triggered control of surface properties provided by different external stimuli or by the immobilization of instructive biomolecular cues has found application in the design of new biomaterials.

10.2.1 Polymer surfaces with tunable cell attachment and detachment

Depending on the application, the surface of the biomaterial should promote cell adhesion (e.g., in an implant) or promote cell detachment (e.g., in cell sheet engineering) (Gil and Hudson, 2004; Roy *et al.*, 2010). Responsive surfaces with cell adhesive properties which are switchable in response to an external stimulus have been extensively applied in the design of smart systems for TE.

Temperature

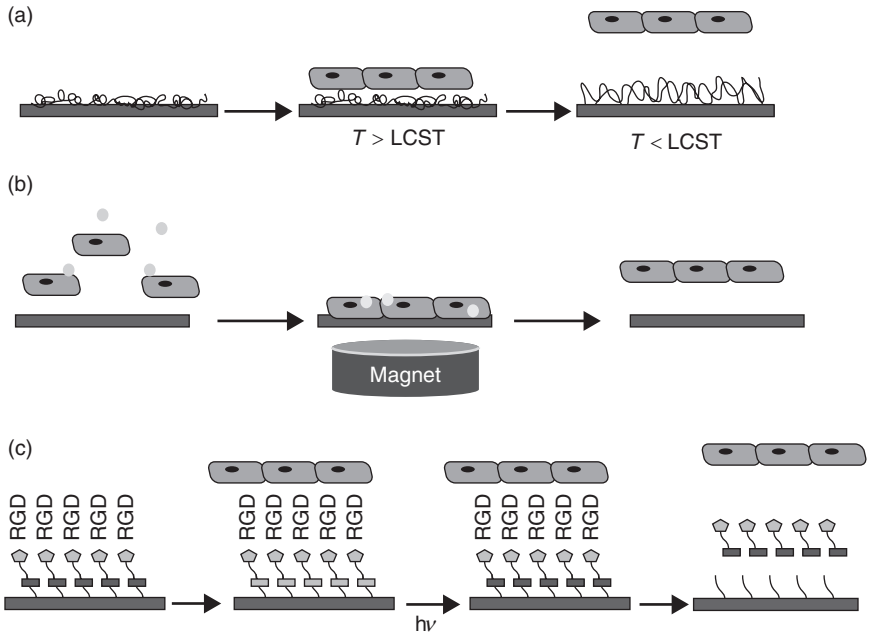
The most popular examples of switchable substrates are the thermosensitive surfaces that change from hydrophobic to hydrophilic substrates upon small temperature changes. Thermoresponsive polymers, which exhibit a lower critical solution temperature (LCST) in water close to body temperature, are the mostly used responsive materials to produce surfaces with tunable cell attachment/detachment (da Silva *et al.*, 2007).

Poly(*N*-isopropylacrylamide) (PNIPAAm), a thermoresponsive polymer whose LCST it is around 32°C, has been frequently used in the fabrication of thermoresponsive surfaces (Kubota *et al.*, 1990). As long as 20 years ago Professor Okano and colleagues demonstrated innovative substrates for cell culture using PNIPAAm-grafted surfaces (Yamada, 1990; Takei *et al.*, 1994; Yakushiji *et al.*, 1998). Electron beam radiation was used to graft various materials including glass, metals, and plastics with PNIPAAm, yielding localized temperature-responsive surfaces. Below the LCST, surfaces are slightly hydrophobic, and cell adhesion and proliferation proceed as in conventional culture conditions. Cultured confluent cell sheets spontaneously detach by reducing the temperature when the polymer becomes insoluble and collapses. These grafted substrates were used for cell culture at 37°C. Cells cultured on such thermoresponsive substrates can be recovered as confluent cell sheets, while keeping the newly deposited extracellular matrix intact by lowering the temperature as a consequence of the hydrophilicity change (see Fig. 10.1a)

Today a large number of reported works have confirmed the relevance and applications of this strategy in TE for the regeneration of several tissues, and a number of clinical trials have already started (Elloumi-Hannachi *et al.*, 2010; Haraguchi *et al.*, 2012; Iwata *et al.*, 2009; Nishida *et al.*, 2004a; Ohki *et al.*, 2006; Takagi *et al.*, 2010). Complex surface designs including micro-patterned domains that can be switched by changing the temperature allowed spatio-temporally control of cell attachment. This strategy has been applied to generate micro-patterned cell co-cultures in combination with cell sheet engineering (Tsuda *et al.*, 2006, 2007).

An alternative to grafted PNIPAAm surfaces, which requires previous activation of the supporting substrate, is offered by multilayer polymer assemblies, such as chitosan-grafted-PNIPAAm and alginate multilayered films (Martins *et al.*, 2011). The layer-by-layer approach allows mild conditions for fabrication and precise control over the thickness of the coatings, which is a relevant issue for modulating cellular adhesiveness.

Elastin-like recombinamers (ELRs) – a recombinant class of elastin-like polymers (ELPs) – are genetically engineered, peptide-based materials that mimic the structure of natural elastin. Although often associated with temperature-responsive polymers, ELRs could be classified as a multiple-responsive material, and surfaces modified with these macromolecules can respond to temperature, pH and ionic strength at the same time (Costa *et al.*, 2011). Smart thin coatings using an ELR containing specific peptide sequences were fabricated through simple deposition of the ELR dissolved in aqueous-based solutions onto different substrates (Costa *et al.*, 2009; Zhang *et al.*, 2006). The conformational changes of the ELR at its inverse temperature transition modified the topography at the nanoscale and the wettability of the polymeric substrate, which could be potentially used to control cell adhesion.



10.1 Schematic representation of systems to control cell attachment/detachment. (a)Temperatures above the LCST allow adsorption of proteins and cell attachment. Cooling below the LCST allows release of cells. (b) Magnetite cationic liposomes are used to label cells that are attracted to the surface by magnetic force. Upon removal of the magnet, cell sheets detached from the surface. (c) A bioactive ligand is attached to the surface mediating cell attachment. Upon light exposure, the chromophore is photocleaved and the linker, along with its target, is removed from the surface leading to cell detachment. (*Source*: Adapted from (Wirkner *et al.*, 2011a).)

Magnetic field

A different approach to cell sheet engineering is the magnetic harvesting of cell sheets using magnetite-containing cationic liposomes (see Fig. 10.1b) (Akiyama *et al.*, 2010; Ito *et al.*, 2005; Shimizu *et al.*, 2007). Magnetite cationic liposomes are used to label mesenchymal stem cells in culture and a magnet is placed under the plate. Cells attracted to the surface by magnetic force are cultured constructing a cell sheet. Upon removal of the magnet, cell sheets detached from the culture surface and could be harvested without enzymatic treatment.

Light

Photosensitive adhesive ligands anchored to a surface have been also used to trigger cell attachment and release from surfaces using light. Recent

works reported the use of photolabile compounds to create surfaces with switchable cell adhesion. This strategy relies on the use of photocleavable group attached to a cell adhesive ligand for light-triggered cell attachment or to a surface linker for light-triggered cell detachment.

Two different strategies have been reported. In the first, photolabile caged adhesive peptides become active and mediate cell adhesion after light exposure (Ohmuro-Matsuyama and Tatsu, 2008; Petersen *et al.*, 2008; Wirkner *et al.*, 2011b). These peptides allow directed cell adhesion in selected regions by site-specific irradiation using masks or scanning lasers. In the second, photocleavable linkers between the cell adhesive sites and the supporting substrate promote cell release from the surface upon irradiation (Wirkner *et al.*, 2011a) (see Fig. 10.1c). Light has several advantages as stimulus source: it can be precisely spatially and temporally controlled and allows modulation of the stimulus by tuning the irradiation time or intensity.

10.2.2 Instructive surfaces with covalently immobilized bioactive molecules

Surface properties of an implantable device are of critical importance since the first contact with the organism is mediated by the interface. This interaction is what drives the subsequent tissue and cellular events, including protein adsorption, cell adhesion, and inflammatory response (Castner and Ratner, 2002; Cole *et al.*, 2009; Stevens and George, 2005). Thus, instructive biointerfaces will first dictate the type of cell that attach and instruct the cell behavior through the motifs presented. Scaffold materials can be functionalized with bioactive molecules, to influence cell adhesion and regulate cell function, either by physical adsorption of specific biomolecules or by chemical immobilization.

Studies have revealed that specific cells can show a preference for adhering to functional biomaterials, with instructive interfaces. For example peripheral blood contains specific cell types with high potential to differentiate through a specific lineage, including circulating cells with osteogenic (Eghbali-Fatourechi *et al.*, 2005) or angiogenic potential (Asahara *et al.*, 1997). For advanced cellular therapies and TE processes, the selection of the required cell type from a mixed cell population is a necessary procedure for a successful treatment. The most studied platform for cell recruitment is the use of covalently immobilized antibodies to isolate specific and rare cell types required for successful regeneration of particular tissues (Bichsel *et al.*, 2012; Hatch *et al.*, 2011; Ye *et al.*, 2009). Recent studies showed how different biomolecules could selectively capture cells from circulating body fluids to an implantable material (Camci-Unal *et al.*, 2010; Meyers *et al.*, 2011; Ye *et al.*, 2009).

Endothelial progenitor cells (EPCs) are a particular cell type of extreme importance in the regeneration of endothelial and cardiac tissue. Current evidence suggests that EPCs may be recruited by the use of specific molecules such as small peptides or antibodies (Camci-Unal *et al*., 2010, 2012; Hatch *et al*., 2011). This strategy could help to treat cardiovascular injuries using circulating EPCs to accelerate the re-endothelialization process of artificial grafts and other related cardiovascular TE applications.

10.3 Instructive hydrogels with a physicochemical response

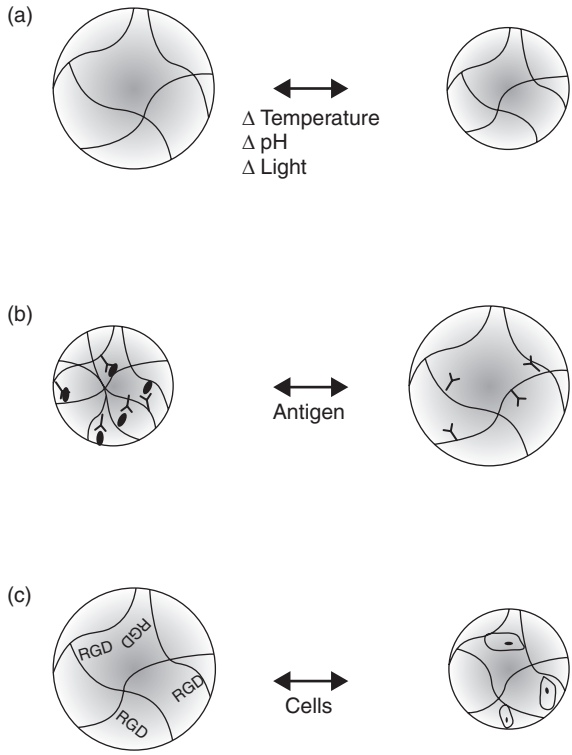
Hydrogels are polymeric networks able to swell in water and build up polymer matrices with tissue-like mechanical properties that can be used as biomaterials for direct encapsulation of cells. In the past, the hydrogel provided a homogeneous environment for the cells and did not allow any control in the temporal and spatial variability of its properties. However, responsive hydrogels have changed this view. These materials experience reversible or irreversible changes in physical and/or chemical properties when subjected to small environmental influences, mainly (but not exclusively) in temperature or pH (Delair, 2012; Mano, 2008). Hydrogels which respond to particular biological components such as enzymes antibodies or cells (Hu *et al.*, 2012; Ulijn, 2006) also constitute a promising strategy for the design of novel bioresponsive materials.

10.3.1 Triggered cross-linking and swelling

Temperature

Various strategies have been proposed for the synthesis of responsive hydrogels for applications in TE and drug delivery (see Fig. 10.2). Among the responsive polymers used for TE applications, temperature-responsive hydrogels have attracted the most interest (Ruel-Gariepy and Leroux, 2004). These polymers show a decrease in solubility that is attributed by changes in the overall hydrophilicity of the polymer chains upon temperature change.

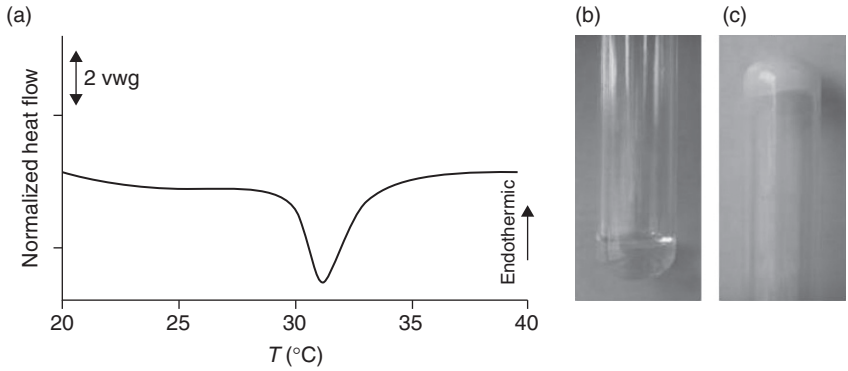
In general, naturally occurring polymers do not exhibit thermal responsiveness. Various strategies were envisioned to confer this physicochemical property to polysaccharides, such as chemical modification or formation of interpenetrated networks with other thermoresponsive polymers. Most of the published works report the use of thermoresponsive hydrogels based on PNIPAAm (Rzaev *et al.*, 2007). Examples of copolymers which may be grafted with PNIPAAm are chitosan (Martins *et al.*, 2011), dextran (Huang and Lowe, 2005; Huh *et al.*, 2000), gelatin (Ohya and Matsuda, 2005), or



10.2 Examples of triggered cross-linked hydrogels. (a) Temperature, pH, and light-triggered sol–gel transition. (b) Polymeric chains with grafted antibodies are cross-linked in the presence of specific antigens. (c) Polymeric chains with immobilized RGD are cross-linked in the presence of cells.

methylcellulose (Liu *et al.*, 2004; Sá-Lima *et al.*, 2011). Figure 10.3 shows the response of PNIPAAm-methylcellulose copolymer at room temperature and 37°C. The phase transition from a hydrophilic to a hydrophobic structure is clearly detected by a shift from a transparent solution at room temperature to an opaque self-sustained gel at 37°C (Fig. 10.3b) (Sá-Lima *et al.*, 2011). Recently, a copolymer synthesized by grafting PNIPAAm onto aminated alginate was also exploited as a thermoresponsive system. *In vitro* results revealed effective cell encapsulation preserving cell viability and promising great potential for application as a cell carrier for TE (Tan *et al.*, 2012).

A different candidate is Pluronic, an amphiphilic triblock copolymer of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide), (PEO-PPO-PEO) (Frisman *et al.*, 2011a,b; Fusco *et al.*, 2006; Ni *et al.*, 2009; Shachaf *et al.*, 2010;).



10.3 PNIPAAm was grafted to methylcellulose using APS as initiator and tetramethylethylenediamine (TEMED) as accelerator. (a) Differential scanning calorimetry (DSC) heating thermogram of the copolymer. Images of the copolymer at room temperature (b) sol and 37°C (c) gel. (*Source:* Reprinted and adapted from Sá-Lima *et al.*, 2011, copyright 2011, with permission from John Wiley and Sons.)

In particular, Pluronic F127 with a LCST around 30°C has been widely studied for TE application. Upon heating to 30°C, the copolymer chains self-assemble and form a micellar (insoluble) aggregation state. Like PNIPAAm, Pluronic 127 has also been conjugated with polymers of natural and synthetic origin to improve the biocompatibility and drug delivery potential. For example injectable hydrogels have been designed by grafting Pluronic onto chitosan using carbodiimide chemistry (Park *et al.*, 2009). The conjugated polymeric solution turns into a hydrogel upon heating above 25°C suggesting the potential of this material as an injectable system for tissue regeneration. In another approach, Han and collaborators synthesized a composite hydrogel of Pluronic F127 derivates and hyaluronic acid (HA) with incorporated transforming growth factor-beta for cartilage regeneration (Jung *et al.*, 2010). Thermosensitive hydrogels are especially attractive as injectable biomaterials due to their spontaneous gelation at body temperature. Injectable scaffolds have been extensively investigated for applications in tissue regeneration as they can be introduced in the body together with cells or growth factors using minimally invasive procedures.

pH

pH-responsive polymers undergo solubility, volume, configurational, or conformational reversible changes in response to external pH (Dai *et al.*, 2008) and have also been extensively applied for biomedical applications (Ghandehari *et al.*, 1996; Gil and Hudson, 2004; Gupta *et al.*, 2002).

Variations in pH are known to occur at several body sites or due to some specific pathologies, making these systems extremely valuable for TE and drug delivery approaches. Unlike temperature changes, the pH change can be exploited for a direct response at a certain tissue or in a cellular compartment. pH-responsive hydrogels are made of chemically or physically cross-linked polymeric backbones that contain pendant acidic or basic groups displaying significant differences in swelling properties depending on the pH of the environment. They mainly contain carboxylic, phosphoric, or amino groups, which are susceptible to protonation/deprotonation, with consequential changes in the volume of a hydrogel (Ghandehari *et al.*, 1996; Tonge and Tighe, 2001).

pH-responsive synthetic polymers like poly(acrylic acid) (PAAc) (Serra *et al.*, 2006; Li *et al.*, 2007) and poly(methacrylic acid) (PMAAc) (Kim *et al.*, 2003) and natural-based polyelectrolytes like chitosan (El-Sherbiny, 2010), alginate (Chan and Neufeld, 2009), HA (Tan *et al.*, 2010) and carrageenans (Kulkarni *et al.*, 2012) have been used in a wide range of biomedical applications. In some cases it is interesting to combine pH-responsive material based polymers with temperature-responsive macromolecules to expand the functionality of the systems (Prabaharan and Mano, 2006).

Light

Light-triggered systems have received a lot of attention due to the non-invasiveness of this trigger, which lead to a variety of elegant structures. Organic molecules whose structure or conformation can be regulated by light have been widely used as 'light switches' to develop photoresponsive functional materials (Mayer and Heckel, 2006). Specifically, photoresponsive hydrogels containing azobenzene moieties have been reported (Sakai *et al.*, 2007; Zheng *et al.*, 2004). Upon irradiation the azobenzene group undergoes a *cis*-to-*trans* isomerization that is accompanied by a change in the polarity of the polymeric material (El Halabieh *et al.*, 2004). For example, a system based on azobenzene-branched PAAc copolymer has been investigated and found to undergo reversible gelation triggered by light (Zhao and Stoddart, 2009). These reversible sol–gel phase transitions of the supramolecular complex can be controlled under these mild conditions, suggesting that this gel material could have a promising role to play in bioengineering applications such as TE.

Another group of photoresponsive hydrogels is based on the spiropyran chromophore. Spiropyrans are non-polar but undergo light-induced isomerization when exposed to UV light and generation of a charged 'open' merocyanine isomer (Sumaru *et al.*, 2006; Szilagyi *et al.*, 2007). Qiu and coworkers developed a photoresponsive hydrogelator in which the spiropyran unit was converted to the corresponding merocyanine form (Qiu *et al.*, 2009). The

gel–sol transition occurs upon exposure of the gel to visible light irradiation, and the gel phase can be regenerated by further UV light irradiation. Results suggest the ability of the system to be used as a biomaterial due to the biocompatibility of the wavelengths used.

Biomolecules

Antigen–antibody interactions have also been explored as triggered cross-linkable hydrogels (Lu *et al.*, 2003; Miyata *et al.*, 1999b). An antibody binds with high affinity and specificity to an antigen through multiple noncovalent bonds. Binding between the antigen and the antibody introduces reversible cross-links in the gel network. This molecular recognition function can be exploited for the design of antigen-sensitive hydrogels that undergo swelling changes in response to a specific antigen (see Fig. 10.2b).

Antigen–antibody binding has been used to induce responses in polymeric systems prepared either by physically entrapping, or chemical conjugation, of antibodies or antigens or using antigen–antibody pairs as reversible cross-linkers within networks. Miyata and colleagues prepared antigen-sensitive polymeric chains by coupling an antigen to an acrylate backbone. The modified monomer was polymerized in the presence of antibodies resulting in the formation of a hydrogel cross-linked both covalently and by antigen–antibody interactions (Miyata *et al.*, 1999a, 2009). Upon the addition of a second free antigen, competitive binding of antibodies resulted in loss of the antigen–antibody cross-linkers and swelling of the hydrogel. A different bioresponsive system described by Mooney and coworkers consists of a system able to gel using cells as attachment points of macromolecular segments (Drury *et al.*, 2005). Alginate chains were modified with Arg-Gly-Asp-Ser-Pro (RGDSP) cell adhesive peptides and ionically cross-linked for encapsulating C2C12 myoblasts. Chemical modification of the polymeric chains with an adhesive peptide gives adhesion points to the cells promoting cross-linking of the network (see Fig. 10.2c) (Drury *et al.*, 2005).

10.3.2 Triggered degradation

The fabrication of engineered hydrogels that mimic the invasive characteristics of native provisional extracellular matrices with controllable degradation rate holds great promise in the development of new smart instructive materials (Hu *et al.*, 2012; Hubbell, 1999; Lutolf *et al.*, 2003b). It is important to note that the control of 3D cell migration within a biomaterial plays a critical role in TE (Ulijn *et al.*, 2007). Insufficient cell migration in a scaffold is a major limitation in the formation of complex TE constructs. Thus, the

development of engineered biomaterials that allow dynamic changes in cell migration is a promising property for TE strategies.

Enzymatic degradation

The integration of specific molecules in polymeric backbones has allowed site-selective control of the degradation of hydrogels (see Fig 10.1a). These systems are usually accomplished by using biomaterials that are sensitive to the proteases expressed at the surfaces of migrating cells, including plasmin and the matrix metalloproteases (MMPs). MMPs are a family of enzymes that have many roles including the breakdown of extracellular matrix (ECM) molecules during tissue remodeling and disease. Due to the selectivity and specificity of enzymes, biomaterials can be programmed to respond to a specific enzyme by incorporation of the specific substrate avoiding nonspecific side-reactions (Ulijn, 2006). In order to engineer hydrogels that respond to cell-secreted enzymes, researchers have mostly used oligopeptides as cross-linkers in poly(ethylene glycol) (PEG)-based hydrogels that are cleavable by MMPs (Ehrbar *et al.*, 2007; Turturro *et al.*, 2012). Moreover, in combination with controlled release of human bone morphogenetic protein, bone healing was successfully achieved (Lutolf *et al.*, 2003a).

A system combining the thermoresponsive properties of Pluronic F127 with a PEG-fibrinogen conjugate backbone was reported as a bioactive material susceptible of cell-mediated remodeling (Frisman *et al.*, 2011a, b). PluronicF127 micelles trapped in a dense hydrogel network create predefined features with controllable nanoscale dimensions. These nanostructural features within the hydrogel could regulate cell spreading.

Light degradation

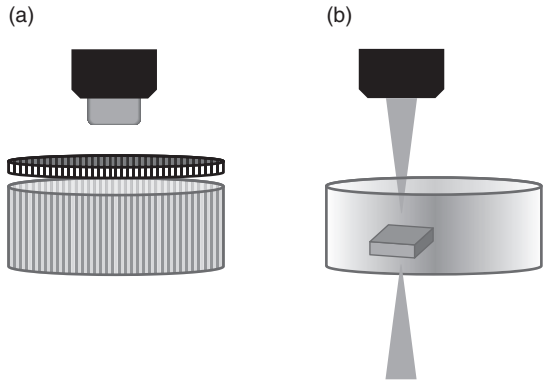
In a different strategy, light was used to control hydrogel degradation in well-defined patterns.

The light-driven changes are mediated by photochemical reactions of a specific functional group (chromophore) contained in the polymer backbone or side chain (Guo *et al.*, 2012; Katz and Burdick, 2010; Shafiq *et al.*, 2012). The chromophore may undergo isomerization, cleavage, or dimerization reactions that cause a change in the polymer chain (Tomatsu *et al.*, 2011). A PEG diacrylate (PEGDA) hydrogel containing Arg-Gly-Asp (RGD) adhesive peptides was modified with photolabile groups that decreased the cross-linking upon light exposure. The change in the cross-linking degree was applied to control cellular migration (Kloxin *et al.*, 2009). A valuable aspect of photosensitive polymeric systems is that light can be applied instantaneously, be focused, and remotely controlled; that is, it is a non-invasive mechanism.

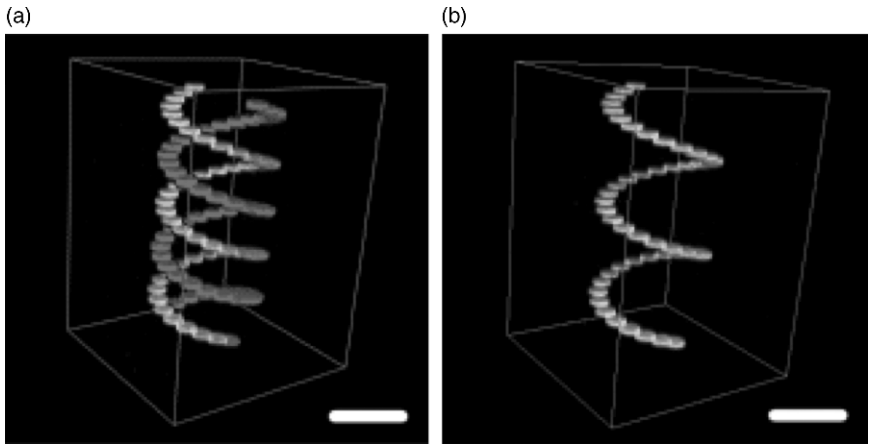
10.4 Materials with 3D defined patterns

Most human tissues are heterogeneous and complex organized structures composed of different cell types. As such, biomaterials for TE applications must be capable of promoting the reconstruction of this heterogenic environment. Hydrogels are typically inert systems and require incorporation of specific signaling molecules to become instructive materials. Cell adhesive peptides and growth factors have been used to modify hydrogels inducing cell attachment and promoting regeneration of tissues (Leipzig *et al.*, 2011; Sun *et al.*, 2011; Zhu, 2010). However, a major drawback of these uniform and static materials is the lack of control in the spatial distribution of bioactive signals required to recapitulate the complexity found in living tissues (Nelson and Tien, 2006). In an attempt to overcome these limitations, recent advances in the design of instructive hydrogels resulted in novel systems that can be modified locally by the incorporation of responsive moieties able to respond to external stimuli creating spatially varying functionalities. Among them, photolabile transparent hydrogels containing moieties that respond to light have been the most studied to create these instructive systems (Kim and Hayward, 2012; Lutolf, 2009). In the past 10 years the introduction of new photopatterning techniques that are still being improved allowed the possibility of patterning bioactive cues within the hydrogel creating well-defined microenvironments (Khetan and Burdick, 2011).

Such techniques allowed significant progress in the field of TE by developing micro-patterned hydrogels that mimic the heterogeneity of native tissue architectures. Different approaches for generating chemically and physically tunable 3D environments in hydrogel for the spatiotemporal regulation of cell behavior have been reported. Most of the approaches are based on photolabile hydrogels whose cross-linking or chemical properties can be locally changed using focused light to build up guidance pathways of biomolecules or physical cues for the cells. Either photomasks or scanning lasers can be used for this purpose, including multiphoton-based approaches for achieving 3D micrometric resolution (see Fig. 10.4) (Culver *et al.*, 2012; Hahn *et al.*, 2006; Lee *et al.*, 2008). For example, Shoichet and collaborators covalently modified agarose gels with a 6-bromo-7-hydroxycoumarin sulfide which, upon exposure to pulsed infrared laser, yielded free thiols (Wosnick and Shoichet, 2008; Wylie and Shoichet, 2011). The free thiols were used to immobilize small peptides that promote cell attachment or growth factors of interest to induce cell differentiation. Anseth and coworkers coupled light-based reactions with copper-free click chemistry to directly encapsulate cells and chemically modify gels avoiding cytotoxic effects (DeForest and Anseth, 2012; DeForest *et al.*, 2009). The reaction is initiated by the thiol addition using visible light and an appropriate photoinitiator followed by a photo-scission of an *o*-nitrobenzyl ether to give a nitroso compound and an



10.4 (a) Schematic representation of the use of a photomask to acquire the desired patterned area where only unmasked regions of the hydrogel undergo light exposure. (b) Using two-photon laser irradiation, light of the appropriate excitation wavelength reaches the focal point, limiting the applied pattern with spatial precision within the gel.



10.5 Thiol-ene patterning of the fluorescent peptide Ac-C-(PL)- Arg-Gly-Asp-Ser-Lys (RGDSK)(AF488)-NH2 into the hydrogel (a) in 3D after exposure to focused pulsed laser light. False coloring is used for enhanced visualization. (b) Subsets of the pre-patterned cues were removed by exposure to UV light to modify the original chemical pattern and yield new 3D patterns. Scale bars = 200 μm. (*Source:* Reprinted and adapted from DeForest and Anseth, 2012, copyright 2011, with permission from John Wiley and Sons.)

acid by-product upon exposure to UV light. This promising method demonstrated that photopatterning techniques can be used for generating reversible changes using bio-orthogonal, light-based biocompatible reactions (see Fig. 10.5).

10.5 Applications in tissue engineering

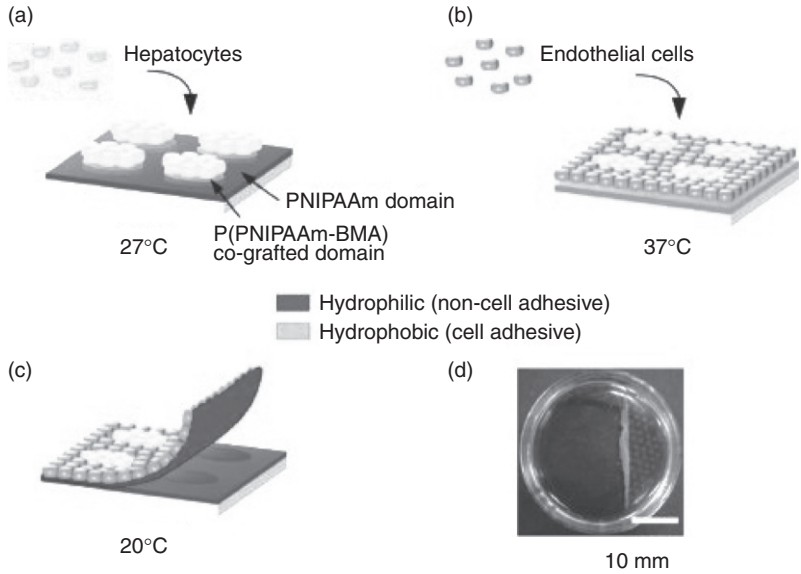
Clinical applications of the systems described in this chapter are still very limited. Nevertheless, it is expected that the intense research effort focused on these technologies will produce significant contributions to make this systems fully available in the clinical market within the coming years. This section highlights the opportunities and promising applications of smart instructive biomaterials in TE.

10.5.1 Cell sheet engineering

The use of surfaces based on smart polymers has been applied to the so-called cell sheet engineering. This technology has been proposed for scaffold-free tissue reconstruction with clinical applications in regenerative medicine (Matsuda *et al.*, 2007; Yang *et al.*, 2005, 2007). Okano and coworkers proposed temperature-responsive cell culture polystyrene substrates prepared by the grafting of PNIPAAm onto these surfaces, which allowed the culture of confluent cell monolayers at 37°C and their recovery as single cell sheets. The patterning of these smart surfaces in well-defined geometries and ordered layering of cell sheets allows the production of complex and organized 3D tissues (Nishida *et al.*, 2004b; Pirraco *et al.*, 2011; Shimizu *et al.*, 2003; Tsuda *et al.*, 2006, 2007). Figure 10.6 shows an example of precisely designed thermoresponsive domains for patterned co-culture of two different cell types. By using a spatial control of surface hydrophobicity, rat primary hepatocytes and bovine carotid artery endothelial cells were sequentially two-dimensionally pattern co-cultured onto the patterned thermoresponsive surfaces. Direct transplantations of cell sheets have been already successfully applied to corneal epithelia (Nishida *et al.*, 2004a), mucosal epithelia (Takagi *et al.*, 2010), periodontal ligament cells (Iwata *et al.*, 2009), and esophageal epithelia (Elloumi-Hannachi *et al.*, 2010; Haraguchi *et al.*, 2012; Ohki *et al.*, 2006).

10.5.2 Smart biomineralization

In bone regeneration it is important to promote the production of bone-like apatite onto the biomaterial surface (Alves *et al.*, 2010). Examples of induced biomineralization found in nature have inspired the production of advanced materials and coatings for a wide range of biomedical and technological applications (Aizenberg, 2004). It has been shown that surface biomimetic mineralization may be triggered by either temperature (Shi *et al.*, 2007) or pH (Dias *et al.*, 2008).



10.6 Schematic representation of method for patterning cell co-culture and harvesting of co-cultured cell sheets using a dually patterned surface. (a) Hepatocytes were seeded and cultured at 27°C, resulting in localization of hepatocytes onto PNIPAAm–*n*-butyl methacrylate (BMA) co-grafted islands showing hydrophobic nature. (b) Endothelial cells were seeded and cultured at 37°C, resulting in generation of patterned co-cultures. (c,d) Decreasing temperature to 20°C induces detachment of co-cultured cell sheet. Harvested patterned co-cultured cell sheet (right). (*Source:* Reprinted and adapted from Tsuda *et al.*, 2006, copyright 2006, with permission from Elsevier.)

10.5.3 Heart valve and vascular graft tissue engineering

The tailoring of biomaterials has been applied to fabricate scaffolds with instructive cues to promote, for example, endothelialization of vascular grafts. TE of heart valves and vascular grafts is a particularly challenging process that requires non-thrombogenic surfaces and fast endothelialization (Bouten *et al.*, 2011; Mendelson and Schoen, 2006). Immobilization on a material surface of antibodies that capture endothelial progenitor cells, preventing attachment of undesired cells, may have an effect on fast endothelialization, thus solving the surface-induced thrombosis and restenosis problem (Fioretta *et al.*, 2012). Antibodies such as anti-CD34 with affinity for endothelial progenitor cells have been immobilized on different surfaces to promote faster endothelialization of vascular grafts (Lin *et al.*, 2010). Recently, Khademhosseini and collaborators successfully developed

HA and heparin-based hydrogels to promote adhesion and spreading of target cells (Camci-Unal *et al.*, 2010). This promising method showed that covalently immobilized CD34 antibody on hydrogels significantly increased the adhesion and spreading of endothelial progenitor cells, at the same time preventing macrophage adhesion. Another study showed the successful application of immobilized CD90 antibodies in decellularized porcine aortic valves to enhance cellularization *in vitro* and *in vivo* even under high shear stress (Ye *et al.*, 2009).

10.5.4 Delivery systems of therapeutic agents

Responsive hydrogels have been successfully used for the controlled delivery of bioactive agents greatly enhancing the efficacy of the regeneration process (Klouda and Mikos, 2008). Swelling and shrinking of temperature- or pH-responsive systems allows the release of certain drugs from the interior in a controlled manner. Common examples of pH variations *in vivo* are found in the gastrointestinal tract or blood stream. Therefore, pH-responsive systems have mainly been developed as carriers systems that allow oral administration or intravenous injection (Kulkarni *et al.*, 2012; Yu *et al.*, 2009).

Thermoresponsive gelatin modified with PNIPAAm was used for the delivery of anionic, cationic, and neutral drugs (Lee and Lee, 2007). Garbern and collaborators developed a pH- and temperature-responsive injectable hydrogel of PNIPAAm-(co-propylacrylic acid-co-butyl acrylate) (Garbern *et al.*, 2011). This polymer forms aqueous solutions at room temperature and pH 7.4, but turns into a gel at 37°C and pH 6.8. This system was investigated for controlled release of angiogenic growth factors in infarcted hearts, where the pH is about 6–7. After 28 days of application, a significant increase in angiogenesis and blood flow in the infarct zone was observed.

Pluronic has been extensively used in the pharmaceutical industry as a carrier for the controlled release of drugs (Kabanov *et al.*, 2002). A copolymer of Pluronic with chitosan was developed as an injectable cell delivery carrier for cartilage regeneration (Park *et al.*, 2009). The efficacy of poly(lactic-co-glycolic acid)/F127 nerve guidance channels has also been demonstrated in an animal trial; the results showed enhanced nerve regeneration associated with the use of this material (Oh *et al.*, 2008).

10.5.5 Hydrogels as injectable implants

Injectable hydrogels undergo a sol–gel phase transition in response to external stimuli, typically a temperature change. At room temperature the polymer is in solution and can be injected in the body to fill in a defect. When

the material reaches body temperature, the swelling ratio decreases and a gel is formed. Innovations in minimally invasive surgery offer several potential advantages over traditional surgical techniques. Injectable hydrogels have shown significant value as a tissue regenerative material, providing moldability to fill a defect using minimal surgical invasion (Tan *et al.*, 2010; Temenoff and Mikos, 2000; Yu and Ding, 2008).

For example, a PNIPAAm-methylcellulose based hydrogel was exploited as an injectable scaffold for cartilage-engineered applications. This copolymer showed a stable gelation at 37°C and entrapped chondrocytes retained their viability, maintaining their phenotype. For cultures up to 28 days the encapsulated cells promoted the accumulation of cartilage-specific ECM (Sá-Lima *et al.*, 2011). Tan and coworkers reported another approach where PNIPAAm-HA hydrogels showed promising results for adipose tissue regeneration (Tan *et al.*, 2009, 2010). Encapsulated human adipose-derived stem cells within hydrogels remained viable, and preliminary *in vivo* studies demonstrated the availability of *in situ* gel formation after injection. These hydrogels appear as promising injectable systems that provide valuable systems to use in minimally invasive TE strategies.

10.5.6 3D scaffolds with spatiotemporal control of biomolecules

3D patterning of growth factors in biomaterials is a key factor to regulate and modify cell and stem cell function (Wosnick and Shoichet, 2008). Two-photon laser scanning of PEGDA hydrogels have also been used to guide encapsulated dermal fibroblasts with precisely patterned RGD moieties (Lee *et al.*, 2008). To recapitulate the dynamic properties that occur *in vivo*, new systems with controllable temporal and spatial variability of biochemical cues have been studied in recent years (DeForest and Anseth, 2011; Kloxin *et al.*, 2009). Photolithographic and multiphoton-based techniques able to design micro-patterns inside a gel will certainly be useful for many applications, creating microenvironments to control cell function.

10.6 Conclusion and future trends

Engineering complex tissues has been the driving force that inspires researchers to design instructive systems with multifunctional properties on demand for tissue regeneration. The designs and applications in TE of the latest and most intensively investigated smart instructive polymers were summarized in this chapter. Tuning stiffness and wettability by the use of responsive polymers, tailoring surfaces with bioactive molecules or designing 3D

patterns within hydrogels at the micro- and nanoscale are the platforms for the design of smart responsive systems herein described. The modulation of smart instructive biomaterials provides instructive environmental cues to surrounding cells, in that way regulating cell adhesion, proliferation, and differentiation towards an effective strategy for tissue regeneration.

Research in smart polymers has undergone tremendous progress in the past few years; however, the effectiveness of these biomaterials as a valuable tool for TE clinical applications is still in its early stages. Major limitations such as rapid and well controllable response, reproducibility, and biocompatibility still need to be overcome. It is crucial to remember that the continuous advances in TE and regenerative medicine will increase the need for more complex and well-organized systems that cannot be obtained by conventional means, and an increased demand for controlled site-specific systems is expected. The rapid progress in the development of the new techniques to engineer the cellular microenvironment will certainly enhance the possibility of a rapid advance toward this objective.

Stimuli responsive polymeric systems in combination with a better design of biomaterials, with control over the chemical and physical properties are critical for an improvement in the efficiency of implantable materials. Consequently, elegant and clever designs by combining smart polymers with micro- and nanopatterned biochemical cues and cells show enormous potential for clinical applicable engineered biomaterials.

10.7 References

Aizenberg, J. (2004). Crystallization in patterns: A bio-inspired approach. *Advanced Materials,* **16**, 1295–1302.

Akiyama, H., Ito, A., Kawabe, Y. and Kamihira, M. (2010). Genetically engineered angiogenic cell sheets using magnetic force-based gene delivery and tissue fabrication techniques. *Biomaterials,* **31**, 1251–1259.

Alves, N. M., Leonor, I. B., Azevedo, H. S., Reis, R. L. and Mano, J. F. (2010). Designing biomaterials based on biomineralization of bone. *Journal of Materials Chemistry,* **20**, 2911–2921.

Asahara, T., Murohara, T., Sullivan, A., Silver, M., Vanderzee, R., Li, T., Witzenbichler, B., Schatteman, G. and Isner, J. M. (1997). Isolation of putative progenitor endothelial cells for angiogenesis. *Science,* **275**, 964–967.

Bichsel, C. A., Gobaa, S., Kobel, S., Secondini, C., Thalmann, G. N., Cecchini, M. G. and Lutolf, M. P. (2012). Diagnostic microchip to assay 3D colony-growth potential of captured circulating tumor cells. *Lab on a Chip,* **12**, 2313–2316.

Bouten, C. V. C., Dankers, P. Y. W., Driessen-Mol, A., Pedron, S., Brizard, A. M. A. and Baaijens, F. P. T. (2011). Substrates for cardiovascular tissue engineering. *Advanced Drug Delivery Reviews,* **63**, 221–241.

Camci-Unal, G., Aubin, H., Ahari, A. F., Bae, H., Nichol, J. W. and Khademhosseini, A. (2010). Surface-modified hyaluronic acid hydrogels to capture endothelial progenitor cells. *Soft Matter,* **6**, 5120–5126.

Camci-Unal, G., Nichol, J. W., Bae, H., Tekin, H., Bischoff, J. and Khademhosseini, A. (2012). Hydrogel surfaces to promote attachment and spreading of endothelial progenitor cells. *Journal of Tissue Engineering and Regenerative Medicine*, **7**(5), 337–347.

Castner, D. G. and Ratner, B. D. (2002). Biomedical surface science: Foundations to frontiers. *Surface Science,* **500**, 28–60.

Chan, A. W. and Neufeld, R. J. (2009). Modeling the controllable pH-responsive swelling and pore size of networked alginate based biomaterials. *Biomaterials,* **30**, 6119–6129.

Cole, M. A., Voelcker, N. H., Thissen, H. and Griesser, H. J. (2009). Stimuli-responsive interfaces and systems for the control of protein-surface and cell-surface interactions. *Biomaterials,* **30**, 1827–1850.

Costa, R. R., Custodio, C. A., Arias, F. J., Rodriguez-Cabello, J. C. and Mano, J. F. (2011). Layer-by-layer assembly of chitosan and recombinant biopolymers into biomimetic coatings with multiple stimuli-responsive properties. *Small,* **7**, 2640–2649.

Costa, R. R., Custodio, C. A., Testero, A. M., Arias, F. J., Rodriguez-Cabello, J. C., Alves, N. M. and Mano, J. F. (2009). Stimuli-responsive thin coatings using elastin-like polymers for biomedical applications. *Advanced Functional Materials,* **19**, 3210–3218.

Culver, J. C., Hoffmann, J. C., Poche, R. A., Slater, J. H., West, J. L. and Dickinson, M. E. (2012). Three-dimensional biomimetic patterning in hydrogels to guide cellular organization. *Advanced Materials,* **24**, 2344–2348.

da Silva, R. M. P., Mano, J. F. and Reis, R. L. (2007). Smart thermoresponsive coatings and surfaces for tissue engineering: switching cell-material boundaries. *Trends in Biotechnology,* **25**, 577–583.

Dai, S., Ravi, P. and Tam, K. C. (2008). pH-Responsive polymers: synthesis, properties and applications. *Soft Matter,* **4**, 435–449.

DeForest, C. A. and Anseth, K. S. (2011). Cytocompatible click-based hydrogels with dynamically tunable properties through orthogonal photoconjugation and photocleavage reactions. *Nature Chemistry,* **3**, 925–931.

DeForest, C. A. and Anseth, K. S. (2012). Photoreversible patterning of biomolecules within click-based hydrogels. *Angewandte Chemie,* **51**, 1816–1819.

DeForest, C. A., Polizzotti, B. D. and Anseth, K. S. (2009). Sequential click reactions for synthesizing and patterning three-dimensional cell microenvironments. *Nature Materials,* **8**, 659–664.

Delair (2012). In situ forming polysaccharide-based 3D-hydrogels for cell delivery in regenerative medicine. *Carbohydrate Polymers*, **87**(2), 1013–1019; Doi: http://dx.doi.org/10.1016/j.carbpol.2011.09.069

Dias, C. I., Mano, J. F. and Alves, N. M. (2008). pH-responsive biomineralization onto chitosan grafted biodegradable substrates. *Journal of Materials Chemistry,* **18**, 2493–2499.

Drury, J. L., Boontheeku, T. and Mooney, D. J. (2005). Cellular cross-linking of peptide modified hydrogels. *Journal of Biomechanical Engineering-Transactions of the Asme,* **127**, 220–228.

Eghbali-Fatourechi, G. Z., Lamsam, J., Fraser, D., Nagel, D., Riggs, B. L. and Khosla, S. (2005). Circulating osteoblast-lineage cells in humans. *New England Journal of Medicine,* **352**, 1959–1966.

Ehrbar, M., Rizzi, S. C., Schoenmakers, R. G., SAN Miguel, B., Hubbell, J. A., Weber, F. E. and Lutolf, M. P. (2007). Biomolecular hydrogels formed and degraded via site-specific enzymatic reactions. *Biomacromolecules,* **8**, 3000–3007.

El Halabieh, R. H., Mermut, O. and Barrett, C. J. (2004). Using light to control physical properties of polymers and surfaces with azobenzene chromophores. *Pure and Applied Chemistry,* **76**, 1445–1465.

El-Sherbiny, I. M. (2010). Enhanced pH-responsive carrier system based on alginate and chemically modified carboxymethyl chitosan for oral delivery of protein drugs: Preparation and in-vitro assessment. *Carbohydrate Polymers,* **80**, 1125–1136.

Elloumi-Hannachi, I., Yamato, M. and Okano, T. (2010). Cell sheet engineering: a unique nanotechnology for scaffold-free tissue reconstruction with clinical applications in regenerative medicine. *Journal of Internal Medicine,* **267**, 54–70.

Fioretta, E. S., Fledderus, J. O., Burakowska-Meise, E. A., Baaijens, F. P., Verhaar, M. C. and Bouten, C. V. (2012). Polymer-based scaffold designs for in situ vascular tissue engineering: controlling recruitment and differentiation behavior of endothelial colony forming cells. *Macromolecular Bioscience,* **12**, 577–590.

Frisman, I., Seliktar, D. and Bianco-Peled, H. (2011a). Nanostructuring PEG-fibrinogen hydrogels to control cellular morphogenesis. *Biomaterials,* **32**, 7839–7846.

Frisman, I., Shachaf, Y., Seliktar, D. and Bianco-Peled, H. (2011b). Stimulus-responsive hydrogels made from biosynthetic fibrinogen conjugates for tissue engineering: Structural characterization. *Langmuir,* **27**, 6977–6986.

Fusco, S., Borzacchiello, A. and Netti, P. A. (2006). Perspectives on: PEO-PPO-PEO triblock copolymers and their biomedical applications. *Journal of Bioactive and Compatible Polymers,* **21**, 149–164.

Garbern, J. C., Minami, E., Stayton, P. S. and Murry, C. E. (2011). Delivery of basic fibroblast growth factor with a pH-responsive, injectable hydrogel to improve angiogenesis in infarcted myocardium. *Biomaterials,* **32**, 2407–2416.

Ghandehari, H., Kopeckova, P., Yeh, P. Y. and Kopecek, J. (1996). Biodegradable and pH sensitive hydrogels: Synthesis by a polymer-polymer reaction. *Macromolecular Chemistry and Physics,* **197**, 965–980.

Gil, E. S. and Hudson, S. M. (2004). Stimuli-responsive polymers and their bioconjugates. *Progress in Polymer Science,* **29**, 1173–1222.

Guo, Q. Y., Wang, X. B., Tibbitt, M. W., Anseth, K. S., Montell, D. J. and Elisseeff, J. H. (2012). Light activated cell migration in synthetic extracellular matrices. *Biomaterials,* **33**, 8040–8046.

Gupta, P., Vermani, K. and Garg, S. (2002). Hydrogels: from controlled release to pH-responsive drug delivery. *Drug Discovery Today,* **7**, 569–579.

Hahn, M. S., Miller, J. S. and West, J. L. (2006). Three-dimensional biochemical and biomechanical patterning of hydrogels for guiding cell behavior. *Advanced Materials,* **18**, 2679–2684.

Haraguchi, Y., Shimizu, T., Yamato, M. and Okano, T. (2012). Scaffold-free tissue engineering using cell sheet technology. *Rsc Advances,* **2**, 2184–2190.

Hatch, A., Hansmann, G. and Murthy, S. K. (2011). Engineered alginate hydrogels for effective microfluidic capture and release of endothelial progenitor cells from whole blood. *Langmuir,* **27**, 4257–4264.

Hu, J., Zhang, G. and Liu, S. (2012). Enzyme-responsive polymeric assemblies, nanoparticles and hydrogels. *Chemical Society Reviews,* **41**, 5933–5949.

Huang, X. and Lowe, T. L. (2005). Biodegradable thermoresponsive hydrogels for aqueous encapsulation and controlled release of hydrophilic model drugs. *Biomacromolecules,* **6**, 2131–2139.

Hubbell, J. A. (1999). Bioactive biomaterials. *Current Opinion in Biotechnology,* **10**, 123–129.

Huh, K. M., Hashi, J., Ooya, T. and Yui, N. (2000). Synthesis and characterization of dextran grafted with poly(N-isopropylacrylamide-co-N,N-dimethyl-acrylamide). *Macromolecular Chemistry and Physics,* **201**, 613–619.

Ito, A., Ino, K., Kobayashi, T. and Honda, H. (2005). The effect of RGD peptide-conjugated magnetite cationic liposomes on cell growth and cell sheet harvesting. *Biomaterials,* **26**, 6185–6193.

Iwata, T., Yamato, M., Tsuchioka, H., Takagi, R., Mukobata, S., Washio, K., Okano, T. and Ishikawa, I. (2009). Periodontal regeneration with multi-layered periodontal ligament-derived cell sheets in a canine model. *Biomaterials,* **30**, 2716–2723.

Jung, H. H., Park, K. and Han, D. K. (2010). Preparation of TGF-beta 1-conjugated biodegradable pluronic F127 hydrogel and its application with adipose-derived stem cells. *Journal of Controlled Release,* **147**, 84–91.

Kabanov, A. V., Batrakova, E. V. and Alakhov, V. Y. (2002). Pluronic((R)) block copolymers for overcoming drug resistance in cancer. *Advanced Drug Delivery Reviews,* **54**, 759–779.

Katz, J. S. and Burdick, J. A. (2010). Light-responsive biomaterials: Development and applications. *Macromolecular Bioscience,* **10**, 339–348.

Khetan, S. and Burdick, J. A. (2011). Patterning hydrogels in three dimensions towards controlling cellular interactions. *Soft Matter,* **7**, 830–838.

Kim, B., LA Flamme, K. and Peppas, N. A. (2003). Dynamic swelling behavior of pH-sensitive anionic hydrogels used for protein delivery. *Journal of Applied Polymer Science,* **89**, 1606–1613.

Kim, J. and Hayward, R. C. (2012). Mimicking dynamic in vivo environments with stimuli-responsive materials for cell culture. *Trends in Biotechnology,* **30**, 426–439.

Klouda, L. and Mikos, A. G. (2008). Thermoresponsive hydrogels in biomedical applications. *European Journal of Pharmaceutics and Biopharmaceutics,* **68**, 34–45.

Kloxin, A. M., Kasko, A. M., Salinas, C. N. and Anseth, K. S. (2009). Photodegradable hydrogels for dynamic tuning of physical and chemical properties. *Science,* **324**, 59–63.

Kubota, K., Hamano, K., Kuwahara, N., Fujishige, S. and Ando, I. (1990). Characterization of Poly(N-Isopropylmethacrylamide) in Water. *Polymer Journal,* **22**, 1051–1057.

Kulkarni, R. V., Boppana, R., Mohan, G. K., Mutalik, S. and Kalyane, N. V. (2012). pH-responsive interpenetrating network hydrogel beads of poly(acrylamide)-g-carrageenan and sodium alginate for intestinal targeted drug delivery: Synthesis, in vitro and in vivo evaluation. *Journal of Colloid and Interface Science,* **367**, 509–517.

Lee, S. H., Moon, J. J. and West, J. L. (2008). Three-dimensional micropatterning of bioactive hydrogels via two-photon laser scanning photolithography for guided 3D cell migration. *Biomaterials,* **29**, 2962–2968.

Lee, W. F. and Lee, S. C. (2007). Effect of gelatin on the drug release behaviors for the organic hybrid gels based on N-isopropylacrylamide and gelatin. *Journal of Materials Science-Materials in Medicine,* **18**, 1089–1096.

Leipzig, N. D., Wylie, R. G., Kim, H. and Shoichet, M. S. (2011). Differentiation of neural stem cells in three-dimensional growth factor-immobilized chitosan hydrogel scaffolds. *Biomaterials,* **32**, 57–64.

Li, S., Yang, Y., Li, H., Yang, X. and Xu, H. (2007). PH-Responsive semi-interpenetrating networks hydrogels of poly(acrylic acid-acrylamide-methacrylate) and amylose. i. synthesis and characterization. *Journal of Applied Polymer Science,* **106**, 3792–3799.

Lin, Q. K., Ding, X., Qiu, F. Y., Song, X. X., Fu, G. S. and Ji, J. (2010). In situ endothelialization of intravascular stents coated with an anti-CD34 antibody functionalized heparin-collagen multilayer. *Biomaterials,* **31**, 4017–4025.

Liu, W. G., Zhang, B. Q., Lu, W. W., Li, X. W., Zhu, D. W., DE Yao, K., Wang, Q., Zhao, C. R. and Wang, C. D. (2004). A rapid temperature-responsive sol-gel reversible poly(N-isopropylacrylamide)-g-methylcellulose copolymer hydrogel. *Biomaterials,* **25**, 3005–3012.

Lu, Z. R., Kopeckova, P. and Kopecek, J. (2003). Antigen responsive hydrogels based on polymerizable antibody Fab ' fragment. *Macromolecular Bioscience,* **3**, 296–300.

Lutolf, M. P. (2009). Biomaterials: Spotlight on hydrogels. *Nature Materials,* **8**, 451–453.

Lutolf, M. P., Lauer-Fields, J. L., Schmoekel, H. G., Metters, A. T., Weber, F. E., Fields, G. B. and Hubbell, J. A. (2003a). Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: Engineering cell-invasion characteristics. *Proceedings of the National Academy of Sciences of the United States of America,* **100**, 5413–5418.

Lutolf, M. P., Raeber, G. P., Zisch, A. H., Tirelli, N. and Hubbell, J. A. (2003b). Cell-responsive synthetic hydrogels. *Advanced Materials,* **15**, 888–892.

Mano, J. F. (2008). Stimuli-responsive polymeric systems for biomedical applications. *Advanced Engineering Materials,* **10**, 515–527.

Martins, G. V., Mano, J. F. and Alves, N. M. (2011). Dual responsive nanostructured surfaces for biomedical applications. *Langmuir,* **27**, 8415–8423.

Matsuda, N., Shimizu, T., Yamato, M. and Okano, T. (2007). Tissue engineering based on cell sheet technology. *Advanced Materials,* **19**, 3089–3099.

Mayer, G. and Heckel, A. (2006). Biologically active molecules with a 'light switch'. *Angewandte Chemie-International Edition,* **45**, 4900–4921.

Mendelson, K. and Schoen, F. J. (2006). Heart valve tissue engineering: Concepts, approaches, progress, and challenges. *Annals of Biomedical Engineering,* **34**, 1799–1819.

Meyers, S. R., Kenan, D. J., Khoo, X. J. and Grinstaff, M. W. (2011). Bioactive stent surface coating that promotes endothelialization while preventing platelet adhesion. *Biomacromolecules,* **12**, 533–539.

Miyata, T., Asami, N. and Uragami, T. (1999a). Preparation of an antigen-sensitive hydrogel using antigen-antibody bindings. *Macromolecules,* **32**, 2082–2084.

Miyata, T., Asami, N. and Uragami, T. (1999b). A reversibly antigen-responsive hydrogel. *Nature,* **399**, 766–769.

Miyata, T., Asami, N. and Uragami, T. (2009). Structural design of stimuli-responsive bioconjugated hydrogels that respond to a target antigen. *Journal of Polymer Science Part B-Polymer Physics,* **47**, 2144–2157.

Yamada, N.,Okano, T., Sakai, H., Karikusa, F.,Sawasaki, Y. and Sakurai, Y. (1990). Thermo-responsive polymeric surfaces; control of attachment and detachment of cultured cells. *Macromolecular Rapid Communications,* **11**, 571–576.

Nair, L. S. and Laurencin, C. T. (2007). Biodegradable polymers as biomaterials. *Progress in Polymer Science,* **32**, 762–798.

Nelson, C. M. and Tien, J. (2006). Microstructured extracellular matrices in tissue engineering and development. *Current Opinion in Biotechnology,* **17**, 518–523.

Ni, X. P., Cheng, A. and Li, J. (2009). Supramolecular hydrogels based on self-assembly between PEO-PPO-PEO triblock copolymers and alpha-cyclodextrin. *Journal of Biomedical Materials Research Part A,* **88a**, 1031–1036.

Nishida, K., Yamato, M., Hayashida, Y., Watanabe, K., Maeda, N., Watanabe, H., Yamamoto, K., Nagai, S., Kikuchi, A., Tano, Y. and Okano, T. (2004a). Functional bioengineered corneal epithelial sheet grafts from corneal stem cells expanded ex vivo on a temperature-responsive cell culture surface. *Transplantation,* **77**, 379–385.

Nishida, K., Yamato, M., Hayashida, Y., Watanabe, K., Yamamoto, K., Adachi, E., Nagai, S., Kikuchi, A., Maeda, N., Watanabe, H., Okano, T. and Tano, Y. (2004b). Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. *The New England Journal of Medicine,* **351**, 1187–1196.

Oh, S. H., Kim, J. H., Song, K. S., Jeon, B. H., Yoon, J. H., Seo, T. B., Namgung, U., Lee, I. W. and Lee, J. H. (2008). Peripheral nerve regeneration within an asymmetrically porous PLGA/Pluronic F127 nerve guide conduit. *Biomaterials,* **29**, 1601–1609.

Ohki, T., Yamato, M., Murakami, D., Takagi, R., Yang, J., Namiki, H., Okano, T. and Takasaki, K. (2006). Treatment of oesophageal ulcerations using endoscopic transplantation of tissue-engineered autologous oral mucosal epithelial cell sheets in a canine model. *Gut,* **55**, 1704–1710.

Ohmuro-Matsuyama, Y. and Tatsu, Y. (2008). Photocontrolled cell adhesion on a surface functionalized with a caged arginine-glycine-aspartate peptide. *Angewandte Chemie-International Edition,* **47**, 7527–7529.

Ohya, S. and Matsuda, T. (2005). Poly(N-isopropylacrylamide) (PNIPAM)-grafted gelatin as thermoresponsive three-dimensional artificial extracellular matrix: molecular and formulation parameters vs. cell proliferation potential. *Journal of Biomaterials Science-Polymer Edition,* **16**, 809–827.

Park, K. M., Lee, S. Y., Joung, Y. K., Na, J. S., Lee, M. C. and Park, K. D. (2009). Thermosensitive chitosan-Pluronic hydrogel as an injectable cell delivery carrier for cartilage regeneration. *Acta Biomaterialia,* **5**, 1956–1965.

Petersen, S., Alonso, J. M., Specht, A., Duodu, P., Goeldner, M. and DEL Campo, A. (2008). Phototriggering of cell adhesion by caged cyclic RGD peptides. *Angewandte Chemie-International Edition,* **47**, 3192–3195.

Pirraco, R. P., Obokata, H., Iwata, T., Marques, A. P., Tsuneda, S., Yamato, M., Reis, R. L. and Okano, T. (2011). Development of Osteogenic Cell Sheets for Bone Tissue Engineering Applications. *Tissue Engineering Part A,* **17**, 1507–1515.

Prabaharan, M. and Mano, J. F. (2006). Stimuli-responsive hydrogels based on polysaccharides incorporated with thermo-responsive polymers as novel biomaterials. *Macromolecular Bioscience,* **6**, 991–1008.

Qiu, Z. J., Yu, H. T., Li, J. B., Wang, Y. and Zhang, Y. (2009). Spiropyran-linked dipeptide forms supramolecular hydrogel with dual responses to light and to ligand-receptor interaction. *Chemical Communications*, 3342–3344.

Ratner, B. D. and Bryant, S. J. (2004). Biomaterials: Where we have been and where we are going. *Annual Review of Biomedical Engineering,* **6**, 41–75.

Roy, D., Cambre, J. N. and Sumerlin, B. S. (2010). Future perspectives and recent advances in stimuli-responsive materials. *Progress in Polymer Science,* **35**, 278–301.

Ruel-Gariepy, E. and Leroux, J. C. (2004). In situ-forming hydrogels – review of temperature-sensitive systems. *European Journal of Pharmaceutics and Biopharmaceutics,* **58**, 409–426.

Rzaev, Z. M. O., Dincer, S. and Piskin, E. (2007). Functional copolymers of N-isopropylacrylamide for bioengineering applications. *Progress in Polymer Science,* **32**, 534–595.

Sá-Lima, H., Tuzlakoglu, K., Mano, J. F. and Reis, R. L. (2011). Thermoresponsive poly(N-isopropylacrylamide)-g-methylcellulose hydrogel as a three-dimensional extracellular matrix for cartilage-engineered applications. *Journal of Biomedical Materials Research Part A,* **98a**, 596–603.

Sakai, T., Murayama, H., Nagano, S., Takeoka, Y., Kidowaki, M., Ito, K. and Seki, T. (2007). Photoresponsive slide-ring gel. *Advanced Materials,* **19**, 2023–2025.

Serra, L., Domenech, J. and Peppas, N. A. (2006). Drug transport mechanisms and release kinetics from molecularly designed poly(acrylic acid-g-ethylene glycol) hydrogels. *Biomaterials,* **27**, 5440–5451.

Shachaf, Y., Gonen-Wadmany, M. and Seliktar, D. (2010). The biocompatibility of Pluronic (R) F127 fibrinogen-based hydrogels. *Biomaterials,* **31**, 2836–2847.

Shafiq, Z., Cui, J. X., Pastor-Perez, L., SAN Miguel, V., Gropeanu, R. A., Serrano, C. and DEL Campo, A. (2012). Bioinspired underwater bonding and debonding on demand. *Angewandte Chemie-International Edition,* **51**, 4332–4335.

Shi, J., Alves, N. M. and Mano, J. F. (2007). Thermally responsive biomineralization on biodegradable substrates. *Advanced Functional Materials,* **17**, 3312–3318.

Shimizu, K., Ito, A., Yoshida, T., Yamada, Y., Ueda, M. and Honda, H. (2007). Bone tissue engineering with human mesenchymal stem cell sheets constructed using magnetite nanoparticles and magnetic force. *Journal of Biomedical Materials Research Part B-Applied Biomaterials,* **82b**, 471–480.

Shimizu, T., Yamato, M., Kikuchi, A. and Okano, T. (2003). Cell sheet engineering for myocardial tissue reconstruction. *Biomaterials,* **24**, 2309–2316.

Stevens, M. M. and George, J. H. (2005). Exploring and engineering the cell surface interface. *Science,* **310**, 1135–1138.

Sumaru, K., Ohi, K., Takagi, T., Kanamori, T. and Shinbo, T. (2006). Photoresponsive properties of poly(N-isopropylacrylamide) hydrogel partly modified with spirobenzopyran. *Langmuir,* **22**, 4353–4356.

Sun, G., Shen, Y. I., Kusuma, S., Fox-Talbot, K., Steenbergen, C. J. and Gerecht, S. (2011). Functional neovascularization of biodegradable dextran hydrogels with multiple angiogenic growth factors. *Biomaterials,* **32**, 95–106.

Szilagyi, A., Sumaru, K., Sugiura, S., Takagi, T., Shinbo, T., Zrinyi, M. and Kanamori, T. (2007). Rewritable microrelief formation on photoresponsive hydrogel layers. *Chemistry of Materials,* **19**, 2730–2732.

Takagi, R., Murakami, D., Kondo, M., Ohki, T., Sasaki, R., Mizutani, M., Yamato, M., Nishida, K., Namiki, H., Yamamoto, M. and Okano, T. (2010). Fabrication of human oral mucosal epithelial cell sheets for treatment of esophageal ulceration by endoscopic submucosal dissection. *Gastrointestinal Endoscopy,* **72**, 1253–1259.

Takei, Y. G., Aoki, T., Sanui, K., Ogata, N., Sakurai, Y. and Okano, T. (1994). Dynamic contact-angle measurement of temperature-responsive surface-properties for poly(N-isopropylacrylamide) grafted surfaces. *Macromolecules,* **27**, 6163–6166.

Tan, H. P., Ramirez, C. M., Miljkovic, N., Li, H., Rubin, J. P. and Marra, K. G. (2009). Thermosensitive injectable hyaluronic acid hydrogel for adipose tissue engineering. *Biomaterials,* **30**, 6844–6853 .

Tan, H. P., Rubin, J. P. and Marra, K. G. (2010). Injectable in situ forming biodegradable chitosan-hyaluronic acid based hydrogels for adipose tissue regeneration. *Organogenesis,* **6**, 173–180.

Tan, R. W., She, Z. D., Wang, M. B., Fang, Z., Liu, Y. S. and Feng, Q. L. (2012). Thermo-sensitive alginate-based injectable hydrogel for tissue engineering. *Carbohydrate Polymers,* **87**, 1515–1521.

Temenoff, J. S. and Mikos, A. G. (2000). Injectable biodegradable materials for orthopedic tissue engineering. *Biomaterials,* **21**, 2405–2412.

Tomatsu, I., Peng, K. and Kros, A. (2011). Photoresponsive hydrogels for biomedical applications. *Advanced Drug Delivery Reviews,* **63**, 1257–1266.

Tonge, S. R. and Tighe, B. J. (2001). Responsive hydrophobically associating polymers: a review of structure and properties. *Advanced Drug Delivery Reviews,* **53**, 109–122.

Tsuda, Y., Kikuchi, A., Yamato, M., Chen, G. P. and Okano, T. (2006). Heterotypic cell interactions on a dually patterned surface. *Biochemical and Biophysical Research Communications,* **348**, 937–944.

Tsuda, Y., Yamato, M., Kikuchi, A., Watanabe, M., Chen, G. P., Takahashi, Y. and Okano, T. (2007). Thermoresponsive microtextured culture surfaces facilitate fabrication of capillary networks. *Advanced Materials,* **19**, 3633–3636.

Turturro, M., Christenson, M., Larson, J. and Papavasiliou, G. (2012). Matrix metalloproteinase (MMP) sensitive PEG diacrylate (PEGDA) hydrogels with spatial variations in matrix properties direct vascular cell invasion. *Journal of Tissue Engineering and Regenerative Medicine,* **6**, 302.

Ulijn, R. V. (2006). Enzyme-responsive materials: a new class of smart biomaterials. *Journal of Materials Chemistry,* **16**, 2217–2225.

Ulijn, R. V., Bibi, N., Jayawarna, V., Thornton, P. D., Todd, S. J., Mart, R. J., Smith, A. M. and Gough, J. E. (2007). Bioresponsive hydrogels. *Materials Today,* **10**, 40–48.

Vacanti, C. A. (2006). The history of tissue engineering. *Journal of Cellular and Molecular Medicine,* **10**, 569–576.

Wirkner, M., Alonso, J. M., Maus, V., Salierno, M., Lee, T. T., Garcia, A. J. and DEL Campo, A. (2011a). Triggered cell release from materials using bioadhesive photocleavable linkers. *Advanced Materials,* **23**, 3907–3910.

Wirkner, M., Weis, S., SAN Miguel, V., Alvarez, M., Gropeanu, R. A., Salierno, M., Sartoris, A., Unger, R. E., Kirkpatrick, C. J. and DEL Campo, A. (2011b). Photoactivatable caged cyclic RGD peptide for triggering integrin binding and cell adhesion to surfaces. *Chembiochem,* **12**, 2623–2629.

Wosnick, J. H. and Shoichet, M. S. (2008). Three-dimensional chemical patterning of transparent hydrogels. *Chemistry of Materials,* **20**, 55–60.

Wylie, R. G. and Shoichet, M. S. (2011). Three-dimensional spatial patterning of proteins in hydrogels. *Biomacromolecules,* **12**, 3789–3796.

Yakushiji, T., Sakai, K., Kikuchi, A., Aoyagi, T., Sakurai, Y. and Okano, T. (1998). Graft architectural effects on thermoresponsive wettability changes of poly(N-isopropylacrylamide)-Modified surfaces. *Langmuir,* **14**, 4657–4662.

Yang, J., Yamato, M., Kohno, C., Nishimoto, A., Sekine, H., Fukai, F. and Okano, T. (2005). Cell sheet engineering: Recreating tissues without biodegradable scaffolds. *Biomaterials,* **26**, 6415–6422.

Yang, J., Yamato, M., Shimizu, T., Sekine, H., Ohashi, K., Kanzaki, M., Ohki, T., Nishida, K. and Okano, T. (2007). Reconstruction of functional tissues with cell sheet engineering. *Biomaterials,* **28**, 5033–5043.

Ye, X. F., Zhao, Q., Sun, X. N. and Li, H. Q. (2009). Enhancement of mesenchymal stem cell attachment to decellularized porcine aortic valve scaffold by in vitro coating with antibody against CD90: A preliminary study on antibody-modified tissue-engineered heart valve. *Tissue Engineering Part A,* **15**, 1–11.

Yu, C. Y., Yin, B. C., Zhang, W., Cheng, S. X., Zhang, X. Z. and Zhuo, R. X. (2009). Composite microparticle drug delivery systems based on chitosan, alginate and pectin with improved pH-sensitive drug release property. *Colloids and Surfaces B-Biointerfaces,* **68**, 245–249.

Yu, L. and Ding, J. D. (2008). Injectable hydrogels as unique biomedical materials. *Chemical Society Reviews,* **37**, 1473–1481.

Zhang, H. L., Iwama, M., Akaike, T., Urry, D. W., Pattanaik, A., Parker, T. M., Konishi, I. and Nikaido, T. (2006). Human amniotic cell sheet harvest using a novel temperature-responsive culture surface coated with protein-based polymer. *Tissue Engineering,* **12**, 391–401.

Zhao, Y. L. and Stoddart, J. F. (2009). Azobenzene-based light-responsive hydrogel system. *Langmuir,* **25**, 8442–8446.

Zheng, P. J., Hu, X., Zhao, X. Y., Li, L., Tam, K. C. and Gan, L. H. (2004). Photoregulated sol-gel transition of novel azobenzene-functionalized hydroxypropyl methylcellulose and its alpha-cyclodextrin complexes. *Macromolecular Rapid Communications,* **25**, 678–682.

Zhu, J. M. (2010). Bioactive modification of poly(ethylene glycol) hydrogels for tissue engineering. *Biomaterials,* **31**, 4639–4656.

11

Smart polymer nanocarriers for drug delivery

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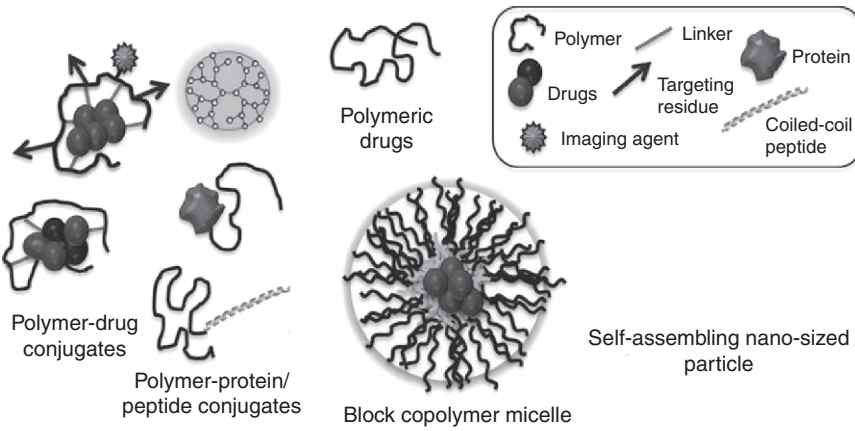
Abstract : Smart polymeric nanocarriers are an important emerging area in drug delivery research. They are capable of releasing their payload in response to a specific stimulus, either present in the body or externally applied. We review the most important and extensively studied stimuli-responsive carriers, with special focus on their *in vitro/in vivo* preclinical evaluation. The most frequently studied stimuli include endogenous pH, enzymes and redox potential. Other emerging applications of externally applied stimuli such as temperature, magnetic field, ultrasound and light are also discussed. Finally, we close with an overall conclusion of the current state of the art and a look towards expected future breakthroughs using this type of nanocarrier.

Key words: stimuli responsive, conjugate, micelle, nanoparticle.

11.1 Introduction

Apart from the identification of new medicines, current pharmaceutical research has focused on macromolecular analogues that are able to improve the therapeutic capabilities of existing drugs by enhancing their biological activity and specificity. To accomplish the full therapeutic potential of a bioactive agent requires targeted delivery. It is crucial to localize therapeutics to the diseased cell and, once there, promote their efficient delivery to the required intracellular compartment and ensure that they are made available for the appropriate duration of time. Nanomedicine can be considered the basis of innovative delivery techniques that offer great potential benefits to patients and new markets for the pharmaceutical industry (Duncan and Gaspar, 2011).

Nanosized drug delivery systems (nanoDDS) have been developed and evaluated. They are considered to be among the most promising approaches to enhancing drug specificity, thereby reducing systemic toxicity (Peer *et al.*, 2007). For the treatment of cancer or other inflammation-related diseases, nanocarriers in general utilize the enhanced permeation and retention



11.1 Schematic representation of the different types of polymer therapeutics. (*Source:* Reproduced from Canal *et al.,* 2011 with permission.)

(EPR) effect[1] (Maeda, 2012). This effect was discovered 30 years ago by Professor Maeda; since then, nanomedicine research has grown exponentially. As a consequence, many nanosized medicines and imaging agents have reached clinical trials (Davis *et al*., 2008; Lammers *et al*., 2008) or are already in routine clinical use (more than 40 nanomedicines are already on the market) (Duncan and Gaspar, 2011). Among them, polymer-based DDS and, in particular, the field coined by Duncan as 'polymer therapeutics' have proven to be one of the most successful nanomedicines (Duncan, 2011; Duncan and Vicent, 2012).

For more details on polymer therapeutics, the reader is referred to a recent special issue of *Advanced Drug Delivery Reviews*, entitled 'Polymer therapeutics: clinical applications and challenges for development' (Vicent *et al*., 2009), and other exhaustive reviews (Canal *et al*., 2011; Duncan, 2011;; Markovsky *et al*., 2012; Sanchis *et al*., 2010). Polymer therapeutics encompasses polymeric drugs, polymer–drug conjugates, polymer–protein conjugates, polymeric micelles (with the drug covalently bound to the polymeric carrier) and polyplexes (designed as non-viral vectors) (Fig. 11.1). Other polymeric nanoDDS include polymeric nanoparticles (Kamaly *et al*., 2012; Kumari *et al*., 2010; Parveen and Sahoo, 2008), nanocapsules (Couvreur *et al*., 2002) and nanospheres (Letchford and Burt, 2007).

[1] The EPR effect occurs in inflamed areas possessing leaky vasculature; this allows preferential extravasation of large molecules (10–200 nm) in the inflamed tissue, and their prolonged retention there owing to the impaired lymphatic drainage (Maeda, 2012).

In order to move towards more advanced nanoDDS, research has been devoted to improving the performance of polymeric carriers by means of designing intelligent, stimuli-responsive nanocarriers (SRNs) (Fleige *et al.*, 2012). Such carriers alter their composition in response to several internal or external stimuli, leading to carrier dissociation and drug release.

In this chapter, the most important, smart, SRNs for drug delivery are described and recent developments in the field are discussed. The most important and commonly used internal stimuli are pH and enzymes (encountered intracellularly as endosomes/lysosomes or in the disease microenvironment). The reduced glutathione (GSH) in the cytosol is also frequently used for the design and synthesis of reduction-sensitive polymeric nanocarriers. Although in the case of difficult-to-reach clinical settings for systemic delivery, thermoresponsive polymers are also used in nanoDDS. They are employed mostly to induce nanoparticle self-assembly by means of a temperature-triggered polymer polarity change, which can be disassembled by external hyper- or hypothermia. Other externally applied stimuli include light, ultrasound and the application of magnetic fields.

11.2 Smart polymeric carriers for drug delivery: pH-responsive nanocarriers

One of the most important stimuli used for the design of smart nanocarriers for drug delivery is pH. It is considered an ideal trigger for selective release of anticancer drugs as the pH in tumours, as well as in areas of inflammation, is lower than the pH of normal tissues (Gerweck and Seetharaman, 1996; Lehner *et al.*, 2012). This can be attributed to the enhanced permeability of the microvessels, the lower perfusion rates and the prolonged transit time of blood through the heterogeneous, disorganized and dysfunctional microvessel network (Fukumura and Jain, 2007). In addition, the vasculature of the tumour is often inadequate to supply the necessary nutrition and oxygen for the expanding population of tumour cells (Ganta *et al.*, 2008). This leads to an increased production of lactic acid by carbonic anhydrase during the anaerobic glycolysis, which also contributes to the acidic microenvironment (Gerweck and Seetharaman, 1996).

Intracellular components like endosomes and lysosomes also normally present a mildly acidic pH (pH 6.5 and 5.5, respectively) (Manchun *et al.*, 2012). This is important for macromolecular DDS, as they are known to be taken up by cells via endocytosis (Mellman, 1996). Drug release can be triggered through the degradation of the polymer main chain and/or through the degradation of pH-sensitive polymer–drug linkers (Mellman, 1996; Mrkvan *et al.*, 2005; Torchilin, 2000). Overall, this greatly contributes to the specificity of the treatment, as drug release is taking place selectively in the

areas of interest. Therefore, the rational design of such systems involves constructing a polymeric nanocarrier that is stable at physiological pH, but will degrade in the acidic environments of tumours or other inflamed areas, where an acidification of the microenvironment occurs, or in the acidic cellular organelles upon cellular uptake.

The design of pH-sensitive nanocarriers involves the application of two principal strategies. In the first of these, the polymer used is pH-sensitive; it either degrades in a pH-sensitive manner, leading to drug release, or its properties are changed (i.e., polarity), resulting in nanocarrier destabilization and concomitant release. In the second strategy, the drug is linked to the polymeric backbone through an acid-cleavable linker.

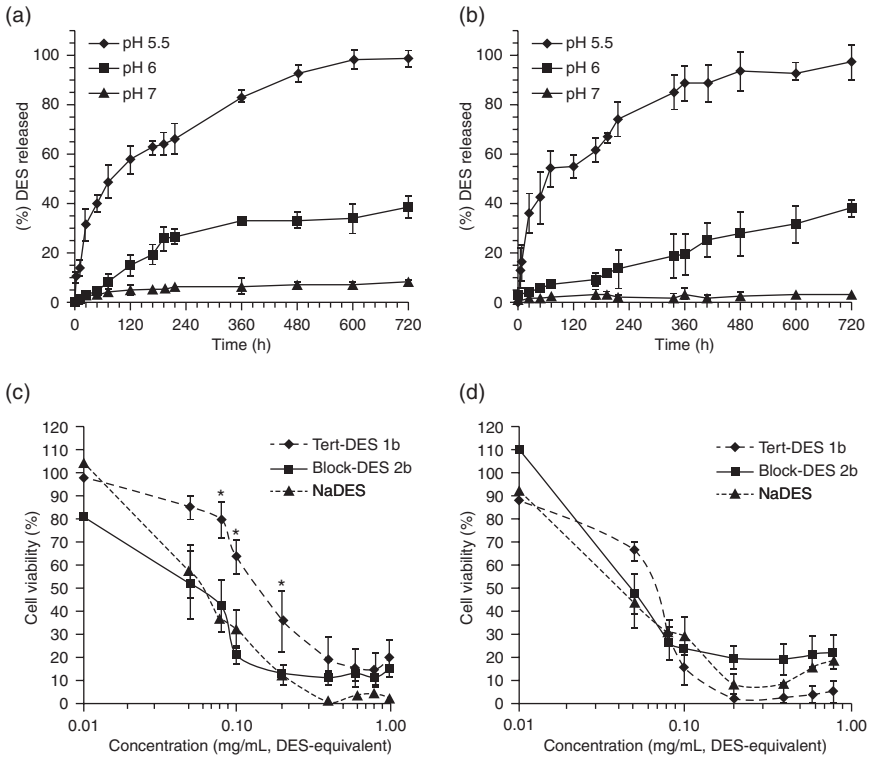
11.2.1 Nanocarriers composed of pH-sensitive polymers

Most of the work on pH-senstitive nanocarriers is based on polymers, which alter their properties upon changes in the pH of the medium. These alterations occur either through hydrolytic degradation or through changes in the physicochemical properties, resulting in the disassembly of the carrier and thus the release of their cargo.

The pioneering work on polymeric systems comprising pH-sensitive polymers was done by Uhrich and co-workers (Erdmann and Uhrich, 2000), with the synthesis and characterization of biodegradable polymeric prodrugs based on salicylic acid (the active component of aspirin) linked to poly(anhydride-ester). This prodrug was found to be stable under acidic conditions (pH 3), but released salicylic acid slowly at normal physiological pH (pH 7.4) and more quickly in basic conditions (pH 10), owing to the hydrolytic degradation of the polymer backbone. This prodrug might show potential in a variety of applications, such as the treatment of gastrointestinal disease, where release in the basic environment of the lower intestine is necessary.

In the same line, polyacetals achieved through the reaction between vinyl ether and alcohols were first described by Heller *et al*. (1980). These systems can be hydrolyzed under mildly acidic conditions (pH 6.5), but are stable at physiological pH (7.4) (Tomlinson *et al*., 2002). Tomlinson *et al*. (2003) demonstrated a prolonged blood half-life and enhanced tumour accumulation for aminopendant polyacetal (APEG)–doxorubicin (DOX) conjugates when compared to the clinical conjugate *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer–DOX, as well as lower liver and spleen uptake.

In vitro cytotoxicity analysis confirmed that the serinol-succinoyl-DOX released during degradation remained active. *In vivo* studies were also performed in B16F10 melanoma-bearing mice. A further step was reported by Vicent *et al*. (2004) when the non-steroidal oestrogen diethylstilboestrol



11.2 pH-dependent DES release from (a) tert-polyacetal-DES at pH 7.4, 6.5 and 5.5, (b) block-polyacetal-DES at pH 7.4, 6.5 and 5.5. Cytotoxicity of tert-polyacetal-DES, block-polyacetal-DES and free DES (as sodium salt, NaDES) after 72 h incubation with PC3 (c) and LNCaP (d) cells. (*Source:* Reproduced with permission from Giménez *et al.*, 2012.)

(DES) was incorporated within the polyacetal mainchain. The first pH-responsive polymeric (tert-DES) drug adequate for lysosomotropic delivery was achieved in this way (Duncan, 2003). These conjugates rapidly released DES at acidic pH (65% in 96 h at pH 5.5) but were stable at neutral environment (4% in 96 h at pH 7.4).

The second generation of DES-polyacetals with more controlled solution conformation has recently been described (Giménez *et al.*, 2012). This includes a family of block polyacetal systems (block-DES), which self-assembled into micellar structures in solution. Both systems, *tert-* and *block*-DES, showed clear pH-dependent drug release kinetics. However, the drug release profile was significantly different with a slightly greater DES release and a 'biphasic' mode for *block*-DES, indicative of the presence of particulate assemblies (Fig. 11.2a,b).

After exhaustive conformational studies using several techniques, including small-angle neutron scattering (SANS) and pulsed gradient spin echo–nuclear magnetic resonance (PGSE-NMR), it was demonstrated that the molecular structure of the conjugate has a significant effect on the solution behaviour, even with the same overall acetal and DES contents. The different release kinetics, together with a greater cell uptake (probably induced by a more spherical shape), yielded an enhancement in cytotoxicity for block-DES in selected prostate cancer cell lines (Fig. 11.2c,d).

Tang and co-workers reported the synthesis and characterization of similar systems based on curcumin-polyacetals (Tang *et al*., 2010), which were shown to be highly cytotoxic *in vitro* against ovarian and breast cancer cell lines (SKOV-3, OVCAR-3 and MCF-7) and *in vivo*, with remarkable antitumour activity in a SKOV-3 ovarian cancer model in mice. Very recently, England *et al*. (2012) confirmed the versatility of these carriers by reporting the first family of nanomedicine (polyacetal–stilbene conjugates) modulators of hypoxia inducible factor-1 (HIF-1), a key transcription factor involved in key cell processes.

Another important class of pH-sensitive systems is the poly(amidoamine)s (PAAs), which undergo hydrolytic degradation of the amidic bonds of their backbone in aqueous media. These have been used as polymer–anticancer drug conjugates and as endosomolytic vectors for intracellular gene and toxin delivery (Ferruti *et al*., 2002). Richardson and co-workers published their results with linear PAAs, which were designed to exhibit minimal non-specific toxicity, display pH-dependent membrane lysis and deliver genes and toxins. *In vitro* and *in vivo* studies using these systems have demonstrated that PAAs are capable of permeabilizing the endocytic vesicular membranes *in vivo*; this is crucial for their use as non-viral gene delivery systems (Richardson *et al*., 2010).

Polyesters are also often used as components of pH-sensitive nanocarriers, owing to their pH-dependent degradation properties. A very important example of such polymers is the polylactides (PLA).These are FDA approved for clinical use; they also have demonstrated biodegradability due to hydrolysis under physiological conditions (Jung-Kwon, 2011). As an example, Ahmed and co-workers blended poly(ethylene glycol) (PEG)-b-PLA and PEG-b-polycaprolactone (PCL) with PEG-b-poly(butadiene) (PEG-b-PBD) and showed that the degradation of the lactic acid block in acidic media resulted in the controlled release of the encapsulated DOX and paclitaxel. This formulation demonstrated shrinkage of rapidly growing tumours upon a single intravenous injection (Ahmed *et al*., 2006).

In another example, biodegradable cationic polymers based on poly(β-amino esters) (PbAE) were used for the development of site-specific drug and gene delivery systems. Under acidic conditions PbAE underwent rapid dissolution, releasing its content. For example, it has been observed that

PEO-modified PbAE (PEO-PbAE) nanoparticles loaded with paclitaxel demonstrate enhanced drug accumulation in the tumour tissue upon intravenous administration in human ovarian adenocarcinoma (SKOV-3) xenografts; there was a significant inhibition of tumour growth in comparison with non-pH-sensitive nanoparticles based on PCL (Devalapally *et al.*, 2007; Shenoy *et al.*, 2005).

pH-responsive nanocarriers can also be constructed from stimuli-responsive polymers that are able to sense small changes in micro-environmental pH, which triggers a corresponding change in physical properties of the polymer such as size, shape or hydrophobicity (Lehner *et al.*, 2012). This behaviour is characteristic of nanoparticles such as those obtained from micellar systems, where the drug is physically encapsulated in the core of the nanocarrier. In these systems, the change in the polymer properties leads to micellar disintegration and subsequent drug release.

Kim *et al.* (2008) synthesized a pH-sensitive mixed-micelle system conjugated with folic acid and with encapsulated DOX, which was designed to treat multidrug resistance (MDR)in cancer. The mixed micelles were composed of poly(histidine)-co-phenylalanine-*b*-PEG (His-co-Phe) and poly(L-lactic acid) (PLLA)-b-PEG-folate. The pH sensitivity of the micelles was conferred by the poly(His-co-Phe) core-forming block, owing to the conversion of histidine from a non-ionized to hydrophilic state by protonation in acidic (endosomal) media; this, in turn, led to micelle destabilization. DOX-loaded micelles demonstrated increased cytotoxicity against both wild-type, sensitive (A2780) and DOX-resistant ovarian MDR cancer cell lines (A2780/DOX^{R}), due to micelle dissociation and DOX release in the endosomes.

In a similar approach, Lee *et al.* (2008) reported on DOX-loaded polymeric micelles consisting of PLLA-b-PEG-b-poly(L-His)-TAT (transactivator of transcription) and poly(L-His)-b-PEG. The micelle core was pH-sensitive owing to the polyHis block, leading to disintegration and DOX release in the acidic endosomes, while the micelles were actively targeted by the attachment of TAT. The micelles were tested *in vivo* in several tumour models (human ovarian drug-resistant A2780/AD, human breast drug-sensitive MCF-7, human lung A549 and human epidermoid KB) and demonstrated increased tumour growth inhibition compared to free DOX and minimum weight loss.

Likewise, the synthesis of micelles based on poly(acrylic acid) (PAA) and poly(methacrylic acid) (PMAc) has been reported for therapeutic use, owing to their ability to swell reversibly with changes in pH. In these cases, the incorporation of ionizable monomer units into polymer backbones leads to pH-dependent phase transitions and solubility changes (Alexander, 2005; Kyriakides *et al.*, 2002). For example, Liu and Armes (2001) described the preparation of pH-sensitive triblock copolymer micelles, synthesized by

atom transfer radical polymerization (ATRP) of glycerol monomethacrylate (GMA) or 2-hydroxyethyl methacrylate (HEMA) and 2-(diethylamino) ethyl methacrylate (DEA), with a poly(ethylene oxide) (PEO) macroinitiator, in order to generate PEO-GMA-DEA or PEO-HEMA-DEA. Both materials were completely soluble at low pH, but above pH 8 DEA was deprotonated, leading to tri-layer micelle formation with a pH-dependent swelling behaviour due to protonation/deprotonation of the DEA core.

pH-sensitive nanocarriers have also been reported from the field of polymersomes, with their conformation and solubility altered by protonation and deprotonation of appropriate functional groups. For example, Checot *et al*. (2003) observed that PBD-b-poly(L-glutamic acid) (PGA) in water is able to form well-defined polymersomes upon addition of NaOH, but the size of the vesicle can be reversibly changed by varying the pH. In addition, UV-cross-linked, pH-sensitive polymersomes have been reported with demonstrated pH-dependent swelling properties, allowing the control of the transmembrane trafficking of encapsulated molecules (Gaitzsch *et al*., 2011, 2012).

11.2.2 Nanocarriers containing pH-sensitive linkers

Another strategy often applied in the design of pH-sensitive nanoconstructs is the responsiveness of the polymer–drug linker. Hydrazone (HYD) ($R_1R_2C{=}NNH_2$) is one of the best known within this family, mostly via pioneering work from Ulbrich's group (Sirova *et al*., 2010). In their studies the behaviour of HYD-linked DOX to HPMA copolymer has been exhaustively described, with important therapeutic efficacy *in vivo* upon injection in tumour-bearing mice (Sirova *et al*., 2010; Ulbrich *et al*., 2003; Chytil *et al*., 2006).

Using HYD as linker Rodrigues *et al*. (1999, 2006) published the synthesis of PEG-DOX and PEG-daunorubicin (DAU) conjugates. These conjugates displayed enhanced *in vitro* activity against human BXF T24 bladder carcinoma and LXFL 529L lung cancer cell lines, whereas on the contrary PEG-DOX conjugates linked through an amide bond showed no *in vitro* anticancer activity, thereby demonstrating the pH-dependent degradation benefits for selective and improved drug delivery.

In a different approach, DOX was linked to the core of core-cross-linked polymeric micelles through a HYD linkage (Talelli *et al*., 2010b). This was accomplished by the incorporation into the micellar core of a DOX methacrylamide derivative (DOX-MA) containing a HYD bond by free radical polymerization. Almost 100% DOX release was demonstrated upon 24 hours of incubation at pH 5 and 37°C, whereas only 5% was released at pH 7.4. *In vitro*, these micelles showed increased cytotoxicity in B16F10 and

OVCAR-3 cells compared to DOX-MA; this was attributed to their cellular uptake via endocytosis and drug release in the acidic endo/lysosomes. *In vivo*, the micelles conferred enhanced antitumour activity and survival over free DOX when injected in B16F10 melanoma-bearing mice.

In addition, the biopolymers hyaluronan (HA) and pullulan have been used for the conjugation of anticancer drugs through pH-sensitive linkers. Cai *et al.* (2010) synthesized HA–DOX conjugates bound via a HYD linkage for treatment of breast cancer. DOX was released in a sustained manner and inhibited breast cancer progression in a xenograft human breast cancer model, resulting in improved survival rates compared to free DOX. Minimal toxicity was observed. Likewise, Zhang *et al.* (2011b) synthesized folate-decorated maleilated pullulan DOX (FA-MP-DOX) conjugate which, *in vitro*, exhibited higher cytotoxicity and enhanced cellular uptake when incubated with ovarian carcinoma A2780 cells, when compared to the free drug.

Another pH-sensitive linker used for the preparation of smart nanocarriers for drug delivery is the cis-aconitic spacer. Choi *et al.* (1999) conjugated DOX to HPMA through this spacer and observed increased release in acidic pH, resulting in significant cytotoxicity upon incubation with human ovarian carcinoma cells.

Interestingly, in a subsequent paper, Ulbrich *et al.* (2003) compared HPMA–DOX conjugates containing DOX bound either through HYD or through a cis-aconitic spacer. A slower release from the cis-aconitic conjugates was observed at pH 5 as compared to the HYD ones, as well as a lower cytotoxicity *in vitro*. Consequently, the aconityl conjugates did not show any therapeutic effect *in vivo*, indicating that drug release from the conjugate occurred but was not sufficient to result in therapeutic efficacy.

11.3 Smart polymeric carriers for drug delivery: enzyme-responsive nanocarriers

An emerging area of research in stimuli-responsive polymeric nanocarriers is related to nanomaterials that experience macroscopic property changes mediated by the catalytic activity of enzymes. The importance of this field is evident by the large number of recent publications related to it (de la Rica *et al.*, 2012; Fleige *et al.*, 2012; Hu *et al.*, 2012).

These systems have a distinctive specificity, as enzymes are highly selective biomolecules. Moreover, enzymes are functional under mild conditions (which is a crucial feature *in vivo*), and are critical components in many biological pathways. For instance, enzymes are key targets for drug development, since they play a key role in cell regulation. Enzymatically sensitive nanomaterials can be designed to deliver and release their cargos specifically at

the target site (Andresen *et al*., 2005; Minelli *et al*., 2010) where the enzyme is over-expressed or its activity is increased due to a pathological situation. Taking into account that up-/down-regulation of enzyme expression or activity is a key aspect in many diseases, enormous benefit can be obtained by the use of enzyme-responsive nanomaterials as delivery systems in the areas of diagnostics and therapeutics.

To date, two main approaches have been adopted for developing enzyme-responsive materials. In the first and most common approach, the nanocarrier itself is sensitive to enzymatic transformation. This is achieved by the use of either an enzymatically degradable polymer or enzymatically sensitive linkers between the drug and the polymer.

The second approach is based on the surface modification of the nanocarriers with molecules that confer enzymatically triggered changes of the physical properties of the carrier solution. This approach is extremely versatile and has been mainly used in developing inorganic enzyme-responsive nanoparticles (Medintz *et al*., 2005; Stevens *et al*., 2004; Zelzer and Ulijn, 2010) for which use as DDS has not been so fully developed because of toxicological concerns. It will not therefore be discussed in this chapter. For further information the reader is referred to some excellent recent literature (de la Rica *et al*., 2012; Ghadiali and Stevens, 2008; Hu *et al*., 2012; Nie *et al*., 2009).

In the rest of this section, key examples of enzyme-responsive nanocarriers will be discussed, as well as their applications for enzyme-mediated drug release.

11.3.1 Nanocarriers based on enzyme-responsive polymers

This category includes polymeric nanocarriers whose structural scaffold presents a responsive behaviour tailored by enzymatic activity, which is achieved by the use of enzymatically degradable polymers. Generally the advantage of using biodegradable polymers is that it allows utilization of higher molecular weight platforms to optimize pharmacokinetics, as they will eventually be degraded into small molecules that can easily be excreted by the body. This is also essential for treatment of diseases that require chronic administration, for example for tissue repair and regenerative medicine (Hardwicke *et al*., 2010; Santamaria *et al*., 2009; Shaunak *et al*., 2004). Dextrins degradable by amylase are considered to be the most promising options.

Among these carriers, systems based on polyglutamates susceptible to degradation by cathepsin B are considered to be the best choices. The lysosomal protease cathepsin B is known to take part in various extracellular

degradation processes (Mort and Buttle, 1997). This enzyme acts primarily as a carboxypeptidase, essentially as peptidyldipeptidase, as with PGA. In the case of PGA as a platform, the PGA–paclitaxel conjugate (Opaxio™) developed by Cell Therapeutics Inc. is the most clinically advanced formulation; it reached clinical trials mainly for non-small-cell lung cancer (NSCLC) and ovarian cancer (Lammers *et al*., 2012), as well as in combination with radiotherapy (Dipetrillo *et al*., 2006) or cisplatin. In this construct, Paclitaxel (PTX) is linked to PGA through an ester bond shielded during blood circulation in order to avoid release triggered by plasma esterases. Interestingly, the therapeutic value of Opaxio™ has been found to be gender-dependent, showing increased survival in women but not in men. The accepted hypothesis is that a correlation between oestrogen levels and cathepsin B activity exists, which was responsible for paclitaxel release. For that reason, cathepsin B is now used as a clinical biomarker in ongoing trials (Wu *et al*., 2012; Zhang *et al*., 2011a).

Adopting this approach, the high level of enzyme specificity might permit, in the near future, the use of personalized therapy, allowing the selection of patients with more possibilities of benefitting from therapy and less probability of suffering from side effects. In addition, the conjugate CT-2106, PGA– camptothecin (CPT), which is based on PGA conjugated to CPT through an ester linkage, has shown enhanced anticancer efficacy against B16 melanoma tumours (Bhatt *et al*., 2003; de Vries *et al*., 2000, 2001; Singer *et al*., 2000, 2001). This conjugate is also in phase II of clinical trials (Singer *et al*., 2001). Both compounds have been developed by Cell Therapeutics Inc.

Hardwicke *et al*. (2008) developed a bioresponsive dextrin-recombinant human epidermal growth factor (rhEGF) conjugate as a polymer therapeutic with potential for use in the promotion of tissue repair. They were able to show in their *in vitro* studies how the novel conjugate was a perfect example of the relatively new concept, polymer-masking-unmasking-protein therapy (PUMPT). As expected, polymer conjugation reduced rhEGF bioactivity, but exposure to physiological concentrations of α-amylase triggered dextrin degradation; this led to protein unmasking with recovery of the bioactivity level seen for unmodified rhEGF. Their proliferation assays using epidermoid carcinoma (HEp2) cells and HaCaT keratinocytes showed that dextrin–rhEGF conjugates, incubated with α-amylase, slowly released rhEGF and restored growth factor activity over an 8-day period (Hardwicke *et al*., 2008). This culminated in enhanced migration/proliferation in normal dermal and chronic wound fibroblasts and keratinocytes *in vitro* (Hardwicke *et al*., 2008, 2010).

These findings were supported in a later *in vivo* study with (db/db) diabetic mice, a well-known model of delayed wound healing. Topically applied dextrin–rhEGF accelerated wound closure and neo-dermal tissue formation

at the macroscopic level, and increased granulation tissue deposition and angiogenesis at the histological level in comparison with untreated mice, or mice treated with succinoylated dextrin and rhEGF controls (Hardwicke *et al*., 2011).

The approach of enzymatically sensitive polymers is also widely used in the case of polymeric micelles that physically encapsulate their cargo and release it upon enzymatic degradation of the polymer of which they are composed. One example of this is the work of Mao and co-workers (Mao and Gan, 2009). They synthesized amphiphilic diblock copolymers based on poly(glycidol-*block*-ε-caprolactone) (PG-*b*-PCL) with well-controlled structure and pendant hydroxyl groups along the hydrophilic block. These copolymers formed 74–95 nm micelles that demonstrated enzymatically triggered release of the encapsulated dye (pyrene) in the presence of lipase, due to degradation of the PCL block, which resulted in micelle dissociation.

The action of several enzymes can also lead to changes in the morphology of polymeric micelles. This has been exploited in the recent work by Ku *et al*. (2011). In this case micelles composed of polymer–peptide block copolymers were prepared, containing substrates either for protein kinase A, for protein phosphatase-1 or for matrix-metalloproteinases (MMP) 2 and 9. They tested the reversible switching of the morphology of these micelles through a phosphorylation/dephosphorylation cycle, which resulted in a reversible change of the micellar size; they also studied the peptide sequence-directed changes in morphology in response to proteolysis. Although this system has not yet been tested as a nanocarrier, it seems to be a good candidate for application in drug delivery, where surface chemistry and morphology are key aspects in the targeting and pharmacokinetics of nanomaterials.

Although outside the scope of this review, from the field of hydrogels, it is worth mentioning that there has been recent interest in systems that degrade under cellular response. For instance, the work of Aimetti *et al*. (2009) describes a PEG hydrogel platform with human neutrophil elastase (HNE) degradable cross-links formed using thiol-ene photo polymerization, which results in a hydrogel degradable at inflammation sites. In this work, rhodamine-labelled bovine serum albumin was entrapped and was successfully released upon treatment with HNE (Aimetti *et al*., 2009).

11.3.2 Nanocarriers containing enzyme-responsive linkers

The use of enzyme-specific linkers between the drug and the carrier has also been extensively studied. The most commonly used enzyme-specific motifs are peptides or sugars whose cleavage mainly relies on the action of esterases or proteases. The cleavage site can be placed next to the conjugated

compound, releasing the free drug, or within the polymer backbone, releasing the linker-drug construct. Among the most extensively studied systems of this kind are linkers susceptible to lysosomal enzymes (exclusively for small drugs). Usually these are oligopeptides specifically designed to be stable in the blood but rapidly cleaved by lysosomal enzymes (cathepsin B or D, and other MMPs), allowing for lysosomotropic drug delivery (Mellman, 1996; Torchilin, 2000). Examples of such oligopeptide linkers include Gly-Phe-Leu-Gly (GFLG) and Gly-Leu-Phe-Gly (GLFG) (Duncan, 2009; Duncan *et al.*, 1983).

There are plenty of reports on linear polymer–drug conjugates that rely on enzymatic cleavage of the linker between the drug and the polymer. The most classic example of this family is PK1 (CT28068), an HPMA-based conjugate with a GFLG linker bound directly to the 3′-amino sugar of DOX (Vasey *et al.*, 1999). This conjugate was the first polymer–drug conjugate to enter clinical trials (Vasey *et al.*, 1999) with a good therapeutic outcome from phase II trials (Duncan, 2009; Seymour *et al.*, 2009). This was followed by HPMA copolymer-DOX-galactosamine conjugate (PK2, CT28069) to confer liver targeting properties in phase I clinical trial in patients with hepatocellular carcinoma (Seymour *et al.*, 2002). Apart from DOX, this polymeric platform with the same linking strategy has been used for the attachment of other bioactive agents, such as platinates (Gianasi *et al.*, 1999, 2002; Loadman *et al.*, 1999) or several drugs simultaneously, to achieve the polymer-based combination therapy concept (Vicent *et al.* 2005; Greco and Vicent, 2009).

The importance of the linking chemistry and the sort of stimuli that trigger the drug release were shown by the work of Kovář and co-workers, where conjugates of HPMA with DOX through a proteolytically degradable (PK1) or by a hydrolytically cleavable (HYD) linker were compared (Kovář *et al.*, 2004, 2007). They demonstrated that the intracellular distribution, anti-proliferative effect and cell death signals of free DOX, HYD and PK1 were clearly different. The mode of action of HYD resembled much more closely that of free DOX, rather than PK1, since HYD showed a high cytostatic activity *in vitro*, induced rapid cell cycle arrest and apoptosis, and affected the expression of selected genes in a comparable pattern to DOX. Interestingly, they found that the PK1 conjugate demonstrated different, and in some cases even opposite, mechanisms of action compared with both free DOX and HYD conjugates, triggering different intracellular pathways involved in the regulation of cell death and proliferation.

In another approach, Segal *et al.* (2009) reported on the synthesis and characterization of a novel conjugate of pHPMA with the potent anti-angiogenic agent TNP-470 and aminobisphosphonate alendronate (ALN), both conjugated through a glycine-glycine-proline-norleucine linker, and cleavable by cathepsin K. Cathepsin K is a cysteine protease over-expressed at resorption

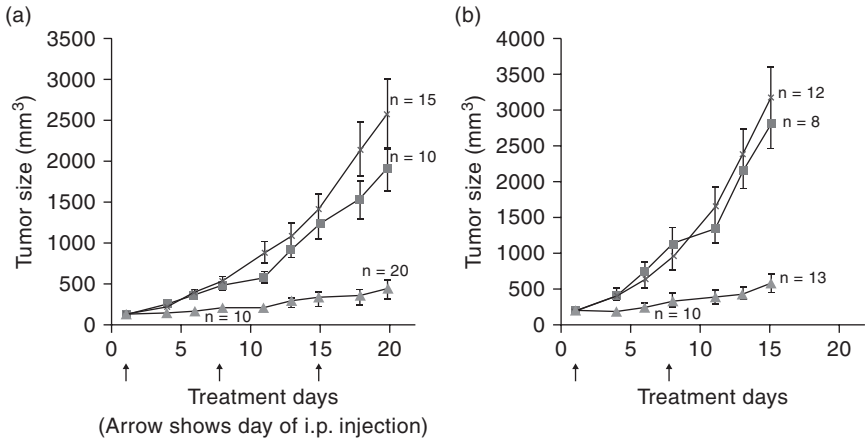
sites in bone tissues, while ALN is considered to be a potent bisphosphonate for the treatment of bone-related diseases and cancer-associated hypercalcaemia. Thus, they were able to synthesize a novel conjugate with synergistic anti-angiogenic and antitumour efficacy. This conjugate showed inhibition of migration, proliferation and capillary-like tube formation of endothelial and human osteosarcoma cells *in vitro*, as well as inhibition of osteosarcoma growth in severe combined inmmunodeficiency (SCID) male mice by 96%.

As the immune system plays a key role in toxicology studies and on tumour progression, the same authors recently reported the assessment of the safety and efficacy profiles of the conjugate using a murine osteosarcoma syngeneic model. The study revealed superior antitumour activity and decreased organ-related toxicities of the conjugate compared to the combination of free ALN plus TNP-470 (Segal *et al*., 2011).

Another example of enzyme-responsive linking chemistry was recently reported by Talelli *et al*. (2011). In this work a novel biodegradable and thermosensitive azide-modified block copolymer based on PEG-*b*-poly[*N*-(2-hydroxypropyl) methacrylamide-lactate] (mPEG-*b*-p(HPMAmLac$_n$)) was synthesized and DOX-glucuronide prodrug (DOX-propGA3) was coupled via click chemistry. The glucuronide spacer of the prodrug used is known to be selectively cleaved by β-glucuronidase, an enzyme present in necrotic tumour areas. Small and monodisperse micelles were formed with this polymer (50 nm) and released 40% of the drug payload after 5 days of incubation at 37°C in the presence of β-glucuronidase, but less than 5% in the absence of the enzyme. DOX micelles incubated with ^{14}C cells showed the same cytotoxicity as free DOX only in the presence of the enzyme, possibly resulting in a system demonstrating tumour-specific drug release.

In Langer's group a novel dextran–peptide–methotrexate conjugate was synthesized to achieve tumour-targeted delivery of chemotherapeutics. The peptide linker was optimized to allow drug release in the presence of matrix-metalloproteinase-2 (MMP-2) and matrix-metalloproteinase-9 (MMP-9), which are two important tumour-associated enzymes. This conjugate (MTX-PVGLIG-dextran) was compared with free methotrexate (MTX) and a control conjugate, containing a non-MMP-sensitive (scrambled, MTX-GIVGPL-dextran) linker, *in vivo*, in two different human tumour models (HT-1080 fibrosarcoma and U-87 glioblastoma) in terms of antitumour efficacy and systemic side effects. It was demonstrated that the MMP-sensitive conjugate showed tolerable *in vivo* side effects and more effective inhibition of *in vivo* tumour growth compared to the free MTX and the MMP non-sensitive construct (Fig. 11.3) (Chau *et al*., 2004, 2006).

Similar approaches have also been used in the field of dendritic polymers. Calderon *et al*. (2009) prepared polymer–drug conjugates from dendritic polyglycerol and maleimide-bearing prodrugs (Tyagarajan *et al*., 2003;



11.3 Tumour growth inhibition of (a) HT-1080 and (b) U-87 tumour-bearing mice upon intraperitoneal injection of free MTX (■), MTX-PVGLIG-dextran (▲), MTX-GIVGPL-dextran (□) and PBS (×) at an MTX dose of 50 mg/kg. The study with MTX-GIVGPL-dextran was stopped at day 6 because of severe toxicity. (*Source:* Reproduced with permission from Chau *et al.*, 2006.)

Warnecke *et al.*, 2007) of DOX and MTX that are cleaved by cathepsin B. A time-dependent drug release was demonstrated in the presence of cathepsin B, and cytotoxicity studies of these conjugates against the human cell lines AsPC1 LN (pancreatic carcinoma) and MDA-MB-231 LN (mamma carcinoma) revealed that the activity of the drugs was essentially retained in the conjugates, most probably owing to intracellular drug release by cathepsin B (Calderon *et al.*, 2009).

Finally, Haba *et al.* (2005) reported the design of dendritic units that release several drug molecules with a single enzymatic cleavage. They prepared the first example of a single-triggered homotrimeric and heterotrimeric prodrug system based on AB3 dendritic units, in which either three molecules of the anticancer drug CPT were attached (homotrimeric system), or CPT, DOX and etoposide (heterotrimeric system), through an enzymatic substrate that is activated from the catalytic antibody 38C2. Both of these trimeric systems were more effective in the inhibition of cell growth when compared to monomeric ones when incubated with the human T-lineage acute lymphoblastic leukaemia cell line MOLT-3, upon activation by the antibody 38C2.

Overall, new advances in polymer chemistry, combined with a growing understanding of basic biological science, are allowing increased development of a new generation of enzyme-responsive polymeric nanocarriers with promising applications in controlled drug release, biocatalysis, imaging, sensing and diagnostics.

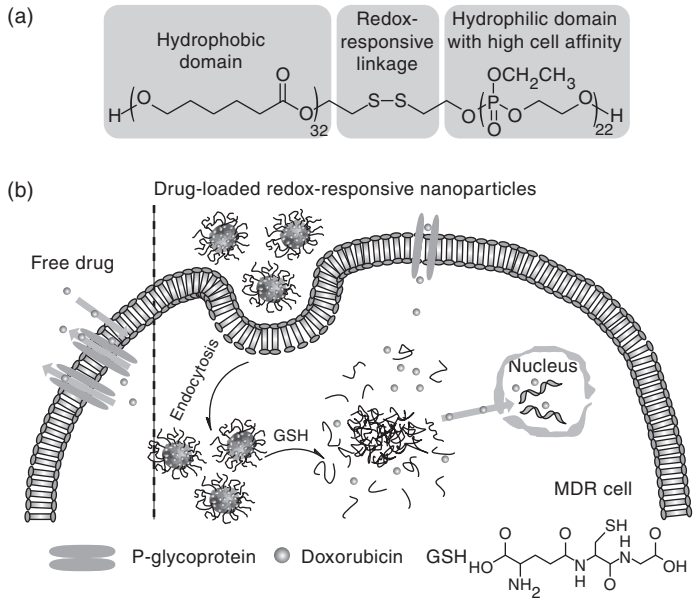
11.4 Smart polymeric carriers for drug delivery: oxidation-responsive nanocarriers

A very important class of SRNs takes advantage of the differences in redox potential in the extra- and intracellular environment (Lehner *et al*., 2012; Meng *et al*., 2009; Roy *et al*., 2010). It has been reported that this difference is 100–1000 fold, with the extracellular space being oxidative, whereas the space inside the cell is strongly reductive. The reductive intracellular environment can mainly be attributed to the presence of high concentrations of glutathione (GSH, 0.5–10 mM), in contrast to the low amounts present in the blood and the extracellular matrices (2–20 μM) (Wu *et al*., 2004). It is therefore obvious that this physiological property can prove very useful for the intracellular release of bioactive molecules. Several polymer therapeutics have been designed for this purpose, including intracellular delivery of DNA, siRNA, proteins and low molecular weight drugs. In addition, reduction-sensitive nanocarriers can prove valuable for tumour-specific drug delivery in cancer therapy because of the highly reducing conditions in the tumour tissue as compared to normal tissues (four-fold higher GSH concentration).

The redox sensitivity of nanocarriers for drug delivery is usually accomplished by disulfide bridges that are either incorporated in the polymer synthetically or are already present on the polymer matrix. These bridges can be cleaved under reducing conditions or in the presence of other thiols (Roy *et al*., 2010). This cleavage results either in the destabilization of the carrier, leading to the release of the encapsulated drug, or to the cleavage of the linker between the carrier and the drug, again liberating the therapeutic, resulting overall in a redox switch.

The most important research on reduction-sensitive nanocarriers has been mainly focused on gene delivery, with the aim of protecting the cargo during transport and enhancing cytosolic delivery. These systems mostly consist of cationic polymers, which are able to complexate DNA and siRNA, and the disulfide cross-linkers that are specifically reduced intracellularly, resulting in the release of the cargo and therefore high transfection efficiency. Since the focus of this chapter is on smart polymers for drug delivery, the reader is referred to some excellent reviews with special focus on the gene delivery field (Fleige *et al*., 2012; Meng *et al*., 2009).

For drug delivery applications, cross-linking through disulfides can be useful for increasing the stability of polymeric micelles or other types of nanoparticles *in vivo*. Wang *et al*. (2011) synthesized disulfide bridged block copolymer of PCL and poly(ethylethylene phosphate) (PCL-SS-PEEP), which self-assembled into 90 nm micellar structures. When these micelles were loaded with DOX, more drug accumulation and retention was observed in MDR cells compared to normal block copolymers, and rapid



11.4 Schematic illustration of (a) the chemical structure of disulfide-bridged PCL-SS-PEEP block copolymer and (b) of the activity of redox-responsive nanocarriers upon cellular uptake and release in the reductive environment of the cytosol.

drug release in the reductive intracellular environment, resulting in higher cytotoxicity. This increased efficacy could be attributed to the intracellular dissociation of the micelles owing to the presence of GSH, leading to DOX release. Figure 11.4 shows a schematic illustration of the activity of this system, which also applies on all reduction-responsive release systems. Sun *et al*. (2011) developed reduction-sensitive micelles based on poly(ethylene oxide)-*b*-poly(*N*-methacryloyl-*N*-(t-butyloxycarbonyl)cystamine) (PEO-b-PMABC) diblock copolymers that were stable in physiological conditions but dissociated under reducing environments, leading to the rapid release of the encapsulated DOX. These micelles demonstrated higher antitumour efficacy compared to micelles without reduction sensitivity against T24 human bladder cancer cells.

Micelles based on thiolated pluronics have also been developed, with disulfide cross-linked cores, loaded with paclitaxel (Abdullah Al *et al*., 2011). These demonstrate accumulation in the cytoplasm and increased cytotoxicity when incubated with A549 cells.

In another interesting approach, Yan *et al*. (2010) prepared polymeric capsules based on disulfide-stabilized PMAc loaded with DOX and studied their interactions with a human primary colorectal carcinoma derived

cell line, LIM1899. A time-dependent cellular uptake of the capsules was observed and an impressive 5000-fold increase in cytotoxicity compared to the free drug.

Another example is the synthesis of the redox-responsive PEG-*b*-poly(lactic acid) (MPEG-SS-PLA) by Song *et al*. (2011) and its use for the preparation of nanoparticles for paclitaxel delivery. Cytotoxicity assays were performed with A549, MCF-7 and HeLa cells; enhanced antitumour efficacy was observed compared to free paclitaxel (empty nanoparticles showed no cytotoxicity), which was attributed to the release of PTX in a triggered and continuous manner owing to the redox sensitivity property.

Reduction-responsive DDS have also been proven valuable for the delivery of proteins in the cytosol. In recent work, caspase 3 was encapsulated in a thin, positively charged polymer shell interconnected by disulfide cross-links. The redox-responsive nanocapsules formed in this way were able to induce apoptosis in several human cancer cell lines, owing to efficient cellular uptake and protein release in the reductive cytosol (Zhao *et al*., 2011).

In summary, very important progress has been made with regard to redox-responsive polymeric nanocarriers during the past decade and it seems that they will soon be among the most promising stimuli-responsive systems (which are currently the pH- and enzyme-responsive systems) when directed to peptide, protein or gene delivery.

11.5 Smart polymeric carriers for drug delivery: temperature-responsive nanocarriers

Another type of SRNs comprises the temperature-responsive polymers (Chilkoti *et al*., 2002; Liu *et al*., 2009; Talelli and Hennink, 2011). The stimuli-responsiveness of these systems is conceptually very different; in most cases it does not serve to enable drug release, but to facilitate self-assembly. Thermosensitive polymers are polymers for which their solutions are characterized by a lower critical solution temperature (LCST). Below this temperature, the polymer chains form hydrogen bonds with water and are therefore in an expanded and fully dissolved state, whereas when heated above this temperature they dehydrate (owing to hydrogen bond cleavage) and become insoluble. This property is extremely useful for the self-assembly of diblock copolymers, consisting of a thermosensitive block (which can be both hydrophilic and hydrophobic, depending on temperature) and a permanently hydrophilic or hydrophobic block, into polymeric micelles – with the thermosensitive polymer serving as the micellar core or corona, respectively. In addition, when these nanoparticles are encapsulating a drug, it is possible to enable drug release by local hypo-/hyperthermia, resulting in a change of the thermosensitive polymer state and dissociation of the

micellar structure. However, the latter has not yet been proven, so this section of the chapter will focus solely on the use of thermosensitivity for polymer self-assembly into nanoscaled carriers.

Among the most commonly used thermosensitive polymers are poly(*N*-isopropylacrylamide) (pNIPAAm) and pluronics (polyethylene glycol-b-polypropylene oxide-b-polyethylene glycol, PEG-*b*-PPO-*b*-PEG). The most extensively studied thermosensitive polymer is pNIPAAm with an LCST of 32°C. This has been used as a hydrophobic block of polymeric micelles with a PEG corona, as well as a hydrophilic shell combined with several other hydrophobic blocks. Work on the synthesis and applications of NIPAAm polymers as components for DDS (in most cases micelles) has been extensively described in several reviews over the years (Aoshima and Kanaoka, 2008; Schmaljohann, 2006; Talelli and Hennink, 2011; Wei *et al*., 2009).

Another well-known thermosensitive polymer often used as a micellar component for drug delivery is pluronics (Kabanov *et al*., 2002; Batrakova and Kabanov, 2008). These triblock copolymers consist of a poly(propylene oxide) middle block flanked by two PEO blocks. They self-assemble into micelles with a PPO block above the LCST of this block. Pluronic micelles loaded with DOX (SP1049C) have reached phase II–III clinical trials, where they have demonstrated slower clearance than the free drug, as well as antitumour activity in advanced MDR adenocarcinoma patients, with acceptable toxicity profile and antitumour activity (Valle *et al*., 2011).

Even though they are very promising, these two polymers display disadvantages in that they are non-biodegradable and also the micelles of these polymers can only disintegrate under local hypo- or hyperthermia to result in controlled drug release, which limits their clinical use. On the other hand, thermosensitive polymers based on *N*-(2-hydroxypropyl)-methacrylamide lactate (HPMAm-Lac_n) have been developed with an LCST that can be easily tailored from 13°C to 65°C by the number of lactate groups attached. Also, most importantly, they degrade over time under physiological conditions (Soga *et al*., 2004a). When this polymer is copolymerizing with PEG, PEG-b-pHPMAmLac_n block copolymers in aqueous solutions self-assemble into small (60 nm) monodisperse micelles; under physiological conditions these micelles disintegrate over time (owing to HPMAmLac_n degradation) leading to drug release (Soga *et al*., 2004b, 2005b). Micelles of diblock copolymers of mPEG-b-pHPMAm-Lac_n have been loaded with several anticancer drugs (DXM, PTX, DOX) and some formulations have already been tested *in vivo* in mice, where they have demonstrated prolonged circulation times, as well as increased antitumour efficacy and animal survival as compared to the free drug (Rijcken *et al*., 2007, 2010; Soga *et al*., 2005a; Talelli *et al*., 2010a).

In summary, thermosensitive polymers can prove very valuable as components of drug delivery systems. This is mainly attributable to the easy preparation of nanoparticles by only changing the solution temperature, which in most cases results in spontaneous self-assembly. However, to confer controlled release properties on these systems, either local hypo- or hyperthermia has to be applied or they need to be combined with another stimulus, such as pH or enzyme sensitivity for tailorable and controlled drug release properties.

11.6 Smart polymeric carriers for drug delivery: nanocarriers responsive to other stimuli

Other than the nanocarriers responsive to the most common stimuli described above, there are various reports in the literature on DDS that release their payload in response to other triggers, such as exogenous light, ultrasound and magnetic field, as well as endogenous glucose and ions. These are described briefly in the following subsections.

11.6.1 Light-responsive nanocarriers

Light-responsive DDS are synthesized by incorporating a moiety that is photo-responsive and, upon irradiation, they release their payload (Fomina *et al*., 2010; Katz and Burdick, 2010). Various polymeric systems with such stimulus-responsiveness have been developed over the years (Katz and Burdick, 2010; Zhao, 2012). The advantages of such a stimulus are its non-invasive nature and the potential for highly precise spatial and temporal application (Fomina *et al*., 2010). However, the patient is required to stay in the dark for some time upon administration, to avoid premature release. The most common photosensitive molecules used are azobenzene (Kumar and Neckers, 1989) and *o*-nitrobenzyl (Zhao *et al*., 2012) groups. Photosensitive moieties containing copolymers have been used to prepare polymeric micelles and vesicles that enable release upon illumination, owing to changes in polarity (Cabane *et al*., 2010, 2011; Tong *et al*., 2005; Wang *et al*., 2004).

11.6.2 Ultrasound-responsive nanocarriers

Ultrasound is another non-invasive trigger that enables drug release from nanocarriers. Even though it is very promising, this application has not been extensively exploited to date. Exposure of a part of the body to ultrasound results in hyperthermia (which might also be useful for the release from the thermally responsive carriers described above) and high- and low-pressure

waves; these generate oscillating bubbles that eventually collapse, resulting in the disruption of the polymer assemblies around them (Husseini and Pitt, 2009; Marmottant and Hilgenfeldt, 2003; Roy *et al.*, 2010).

In addition, there are reports suggesting that, through the application of ultrasound, the degradation times of biodegradable polymers can be increased (Kost *et al.*, 1988). As an example, pluronic block copolymer micelles have been tested in combination with ultrasound, and an increased drug release rate was observed (Munshi *et al.*, 1997). Interestingly, ultrasound has also been shown to increase cellular uptake and nucleus internalization of DOX-loaded pluronic micelles (Marin *et al.*, 2001).

In other recent work, Du *et al.* (2011) prepared DOX-loaded PLGA-mPEG nanobubbles, which demonstrated drug release only upon application of ultrasound. In addition, when administered *in vivo* 85% more tumour inhibitory effect was observed in the group treated with the nanoparticles combined with ultrasound, as compared to the nanoparticles without ultrasound (Du *et al.*, 2011).

11.6.3 Magnetic field-responsive nanocarriers

The design of magnetically responsive nanocarriers in most cases involves the use of paramagnetic or superparamagnetic nanoparticles within a polymeric carrier. These nanoparticles are in most cases superparamagnetic iron oxide nanoparticles (SPIONs), which comprise magnetite (Fe_3O_4) or maghaemite (Fe_2O_3) and are sized between 1 and 100 nm (Yigit *et al.*, 2012). Because of their low toxicity and their high relaxation times, they have attracted a lot of attention as magnetic resonance imaging (MRI) contrast agents, as an alternative to gadolinium-based agents (Wang *et al.*, 2001). Lately, however, their possible applications in drug delivery have been revealed and a lot of research has been directed towards them (Pankhurst *et al.*, 2003).

Drug-loaded SPIONs have been investigated for magnetic drug targeting, in which they can be directed to the areas of interest by an external magnetic field, while at the same time their fate can be followed by means of MRI (image-guided drug delivery) (Neuberger *et al.*, 2005). In addition, SPIONs have been shown to produce elevated temperature when exposed to an alternating magnetic field, making them highly useful for cancer destruction by hyperthermia (Kumar and Mohammad, 2011).

This last property has been often utilized recently for the design of magnetic field-responsive nanocarriers, in combination with thermosensitive polymers. As an example, Baeza *et al.* (2012) coated mesoporous silica nanoparticles with pNIPAAm and loaded them with a fluorescent dye and SPIONs. They observed that application of an alternating magnetic field caused release of the dye from the particles, which could be attributed to

the collapse of the pNIPAAm coating because of the heating effect of the SPIONs (Baeza *et al*., 2012). Using this approach, the particles can act as dual therapeutic agents: they cause drug release through the nanocarrier dissociation, combined with killing of cancer cells by hyperthermia. In a similar approach, Hoare *et al*. (2009) prepared nanocomposite membranes composed of pNIPAAm nanogels and SPIONs loaded with a fluorescent dye; they observed pulsed release of the dye upon the application of magnetic cycles.

11.7 Conclusion and future trends

Overall, stimuli-responsive, smart nanocarriers are a very important emerging area of drug delivery. Those most commonly studied involve endogenous stimuli (pH, enzymes and redox potential), which take advantage of specific conditions that exist intracellularly or in the microenvironment of the disease site, enabling selective drug release.

Other than the endogenous stimuli, there is an exponentially growing interest in stimuli that are applied externally, and in most cases using techniques that are already employed in clinics for other purposes, such as magnetic field and ultrasound. Even though this approach is very promising, the main challenge is to coordinate drug accumulation with exogenous stimuli application with adequate force to trigger drug release. For this reason, this approach is commonly combined with imaging techniques to monitor nano-DDS body distribution.

Overall, this chapter has summarized the most recent advances in the development of smart nanocarriers. Most of the work published so far is still at the proof-of-concept level and the *in vivo* efficacy and safety of these nanocarriers still has to be assessed. However, the authors believe it has been clearly shown that these types of carriers have great potential as clinical candidates.

11.8 References

Abdullah Al, N., Lee, H., Lee, Y. S., Lee, K. D. and Park, S. Y. (2011) Development of disulfide core-crosslinked pluronic nanoparticles as an effective anticancer-drug-delivery system. *Macromol Biosci*, **11,** 1264–1271.

Ahmed, F., Pakunlu, R. I., Srinivas, G., Brannan, A., Bates, F., Klein, M. L., Minko, T. and Discher, D. E. (2006) Shrinkage of a rapidly growing tumor by drug-loaded polymersomes: pH-triggered release through copolymer degradation. *Mol Pharm*, **3,** 340–350.

Aimetti, A. A., Machen, A. J. and Anseth, K. S. (2009) Poly(ethylene glycol) hydrogels formed by thiol-ene photopolymerization for enzyme-responsive protein delivery. *Biomaterials*, **30,** 6048–6054.

Alexander, C. (2005) Stimuli responsive polymers for biomedical applications. *Chem Soc Rev*, **34,** 276–285.

Andresen, T. L., Jensen, S. S. and Jorgensen, K. (2005) Advanced strategies in liposomal cancer therapy: Problems and prospects of active and tumor specific drug release. *Progr Lipid Res*, **44,** 68–97.

Aoshima, S. and Kanaoka, S. (2008) Synthesis of stimuli-responsive polymers by living polymerization: poly(N-isopropylacrylamide) and poly(vinyl ether)s. *Adv Polym Sci*, **210**, 169–208.

Baeza, A., Guisasola, E., Ruiz-Hernández, E. and Vallet-Regí, M. (2012) Magnetically triggered multidrug release by hybrid mesoporous silica nanoparticles. *Chem Mat*, **24,** 517–524.

Batrakova, E. V. and Kabanov, A. V. (2008) Pluronic block copolymers: Evolution of drug delivery concept from inert nanocarriers to biological response modifiers. *J Control Release*, **130,** 98–106.

Bhatt, R. L., de Vries, P., Tulinsky, J., Bellamy, G., Baker, B., Singer, J. W. and Klein, P. (2003) Synthesis and in vivo antitumor activity of poly(L-glutamic acid) conjugates of 20(S)-camptothecin. *J Med Chem*, **46,** 190–193.

Cabane, E., Malinova, V. and Meier, W. (2010) Synthesis of photocleavable amphiphilic block copolymers: Toward the design of photosensitive nanocarriers. *Macromol Chem Phys*, **211,** 1847–1856.

Cabane, E., Malinova, V., Menon, S., Palivan, C. G. and Meier, W. (2011) Photoresponsive polymersomes as smart, triggerable nanocarriers. *Soft Matter*, **7,** 9167–9176.

Cai, S., Thati, S., Bagby, T. R., Diab, H.-M., Davies, N. M., Cohen, M. S. and Forrest, M. L. (2010) Localized doxorubicin chemotherapy with a biopolymeric nanocarrier improves survival and reduces toxicity in xenografts of human breast cancer. *JControl Release*, **146,** 212–218.

Calderon, M., Graeser, R., Kratz, F. and Haag, R. (2009) Development of enzymatically cleavable prodrugs derived from dendritic polyglycerol. *Bioorg Med Chem Lett*, **19,** 3725–3728.

Canal, F., Sanchis, J. and Vicent, M. J. (2011) Polymer-drug conjugates as nano-sized medicines. *Curr Opin Biotechnol*, **22,** 894–900.

Chau, Y., Padera, R. F., Dang, N. M. and Langer, R. (2006) Antitumor efficacy of a novel polymer-peptide-drug conjugate in human tumor xenograft models. *Intl J Cancer*, **118,** 1519–1526.

Chau, Y., Tan, F. E. and Langer, R. (2004) Synthesis and characterization of dextran-peptide-methotrexate conjugates for tumor targeting via mediation by matrix metalloproteinase II and matrix metalloproteinase IX. *Bioconjugate Chem*, **15,** 931–941.

Checot, F., Lecommandoux, S., Klok, H. A. and Gnanou, Y. (2003) From supramolecular polymersomes to stimuli-responsive nano-capsules based on poly(diene-b-peptide) diblock copolymers. *Eur Phys J E Soft Matter*, **10,** 25–35.

Chilkoti, A., Dreher, M. R., Meyer, D. E. and Raucher, D. (2002) Targeted drug delivery by thermally responsive polymers. *Adv Drug Deliv Rev*, **54,** 613–630.

Choi, W. M., Kopečková, P., Minko, T. and Kopeček, J. (1999) Synthesis of HPMA copolymer containing adriamycin bound via an acid- labile spacer and its activity toward human ovarian carcinoma cells. *J Bioact Comp Polym*, **14,** 447–456.

Chytil, P., Etrych, T., Konak, C., Sirova, M., Mrkvan, T., Rihová, B. and Ulbrich, K. (2006) Properties of HPMA copolymer-doxorubicin conjugates with pH-controlled activation: effect of polymer chain modification. *J Control Release*, **115,** 26–36.

Couvreur, P., Barratt, G., Fattal, E., Legrand, P. and Vauthier, C. (2002) Nanocapsule technology: A review. *Crit Rev Ther Drug Carr Syst*, **19,** 99–134.

Davis, M. E., Chen, Z. and Shin, D. M. (2008) Nanoparticle therapeutics: An emerging treatment modality for cancer. *Nat Rev Drug Discov*, **7,** 771–782.

de la Rica, R., Aili, D. and Stevens, M. M. (2012) Enzyme-responsive nanoparticles for drug release and diagnostics. *Adv Drug Deliv Rev*, **64,** 967–978.

de Vries, P., Bhatt, R., Stone, I., Klein, P. and Singer, J. (2001) Optimization of CT-2106: A water-soluble poly-L-glutanlic acid (PG) – camptothecin conjugate with enhanced in vivo anti-tumor efficacy. *Clin Cancer Res*, **7,** 3673S.

de Vries, P., Bhatt, R., Tulinsky, J., Heasley, E., Stone, I., Klein, P., Li, C., Wallace, S., Lewis, R. and Singer, J. (2000) Water-soluble poly-(L)-glutamic acid (PG)-camptothecin (CPT) conjugates enhance CTP stability and efficacy in vivo. *Clin Cancer Res*, **6,** 4511S.

Devalapally, H., Shenoy, D., Little, S., Langer, R. and Amiji, M. (2007) Poly(ethylene oxide)-modified poly(beta-amino ester) nanoparticles as a pH-sensitive system for tumor-targeted delivery of hydrophobic drugs: Part 3. Therapeutic efficacy and safety studies in ovarian cancer xenograft model. *Cancer Chemother Pharmacol*, **59,** 477–484.

Dipetrillo, T., Milas, L., Evans, D., Akerman, P., Ng, T., Miner, T., Cruff, D., Chauhan, B., Iannitti, D., Harrington, D. and Safran, H. (2006) Paclitaxel Poliglumex (PPX-Xyotax) and concurrent radiation for esophageal and gastric cancer: A phase I study. *Amer J Clin Oncol: Cancer Clin Trial*, **29,** 376–379.

Du, L., Jin, Y., Zhou, W. and Zhao, J. (2011) Ultrasound-triggered drug release and enhanced anticancer effect of doxorubicin-loaded poly(D,L-lactide-co-glycolide)-methoxy-poly(ethylene glycol) nanodroplets. *Ultrasound Med Biol*, **37,** 1252–1258.

Duncan, R. (2003) The dawning era of polymer therapeutics. *Nat Rev Drug Discov*, **2**, 347–360.

Duncan, R. (2009) Development of HPMA copolymer-anticancer conjugates: Clinical experience and lessons learnt. *Adv Drug Deliv Rev*, **61,** 1131–1148.

Duncan, R. (2011) Polymer therapeutics as nanomedicines: New perspectives. *Curr Opin Biotechnol*, **22,** 492–501.

Duncan, R. and Gaspar, R. (2011) Nanomedicine(s) under the microscope. *Mol Pharm*, **8,** 2101–2141.

Duncan, R. and Vicent, M. J. (2012) Polymer therapeutics-prospects for 21st century: The end of the beginning. *Adv Drug Deliv Rev,* **65**, 60–70..

Duncan, R., Cable, H. C., Lloyd, J. B., Rejmanova, P. and Kopecek, J. (1983) Polymers containing enzymatically degradable bonds, 7. Design of oligopeptide side-chains in poly[N-(2-hydroxypropyl)methacrylamide] copolymers to promote efficient degradation by lysosomal enzymes. *Makromol Chemie-Macromol Chem Phys*, **184,** 1997–2008.

England, R. M., Masiá, E., Giménez, V., Lucas, R. and Vicent, M. J. (2012) Polyacetal-stilbene conjugates – The first examples of polymer therapeutics for the inhibition of HIF-1 in the treatment of solid tumours. *J Control Release,* **164**, 314–322.

Erdmann, L. and Uhrich, K. E. (2000) Synthesis and degradation characteristics of salicylic acid-derived poly(anhydride-esters). *Biomaterials*, **21,** 1941–1946.

Ferruti, P., Marchisio, M. A. and Duncan, R. (2002) Poly(amido-amine)s: Biomedical Applications. *Macromol Rapid Comm*, **23,** 332–355.

Fleige, E., Quadir, M. A. and Haag, R. (2012) Stimuli-responsive polymeric nanocarriers for the controlled transport of active compounds: Concepts and applications. *Adv Drug Deliv Rev*, **64,** 866–884.

Fomina, N., Mcfearin, C., Sermsakdi, M., Edigin, O. and Almutairi, A. (2010) UV and near-IR triggered release from polymeric nanoparticles. *J Am Chem Soc*, **132,** 9540–9542.

Fukumura, D. and Jain, R. K. (2007) Tumor microvasculature and microenvironment: Targets for anti-angiogenesis and normalization. *Microvasc Res*, **74,** 72–84.

Gaitzsch, J., Appelhans, D., Grafe, D., Schwille, P. and Voit, B. (2011) Photo-crosslinked and pH sensitive polymersomes for triggering the loading and release of cargo. *ChemComm*, **47,** 3466–3468.

Gaitzsch, J., Appelhans, D., Wang, L., Battaglia, G. and Voit, B. (2012) Synthetic bionanoreactor: Mechanical and chemical control of polymersome membrane permeability. *Angew Chem Int Ed*, **51,** 4448–4451.

Ganta, S., Devalapally, H., Shahiwala, A. and Amiji, M. (2008) A review of stimuli-responsive nanocarriers for drug and gene delivery. *J Control Release*, **126,** 187–204.

Gerweck, L. E. and Seetharaman, K. (1996) Cellular pH gradient in tumor versus normal tissue: Potential exploitation for the treatment of cancer. *Cancer Res*, **56,** 1194–1198.

Ghadiali, J. E. and Stevens, M. M. (2008) Enzyme-responsive nanoparticle systems. *Adv Mat*, **20,** 4359–4363.

Gianasi, E., Buckley, R. G., Latigo, J., Wasil, M. and Duncan, R. (2002) HPMA copolymers platinates containing dicarboxylato ligands. Preparation, characterisation and in vitro and in vivo evaluation. *J Drug Target*, **10,** 549–556.

Gianasi, E., Wasil, M., Evagorou, E. G., Keddle, A., Wilson, G. and Duncan, R. (1999) HPMA copolymer platinates as novel antitumour agents: In vitro properties, pharmacokinetics and antitumour activity in vivo. *Eur J Cancer*, **35,** 994–1002.

Giménez, V., James, C., Armiñán, A., Schweins, R., Paul, A. and Vicent, M. J. (2012) Demonstrating the importance of polymer-conjugate conformation in solution on its therapeutic output: Diethylstilbestrol (DES)-polyacetals as prostate cancer treatment. *J Control Release*, **159,** 290–301.

Greco, F. and Vicent, M.J. (2009) Combination therapy: Opportunities and challenges for polymer–drug conjugates as anticancer nanomedicines *Adv Drug Deliv Rev*, **61**, 1200–1213.

Haag, R. and Kratz, F. (2006) Polymer therapeutics: Concepts and applications. *Angew Chem Int Ed*, **45,** 1198–1215.

Haba, K., Popkov, M., Shamis, M., Lerner, R. A., Barbas, C. F. and Shabat, D. (2005) Single-triggered trimeric prodrugs. *Angew Chem Int Ed*, **44,** 716–720.

Hardwicke, J., Ferguson, E. L., Moseley, R., Stephens, P., Thomas, D. W. and Duncan, R. (2008) Dextrin-rhEGF conjugates as bioresponsive nanomedicines for wound repair. *J Control Release*, **130,** 275–283.

Hardwicke, J., Moseley, R., Stephens, P., Harding, K., Duncan, R. and Thomas, D. W. (2010) Bioresponsive dextrin-rhEGF conjugates: In vitro evaluation in models

relevant to its proposed use as a treatment for chronic wounds. *Mol Pharm*, **7,** 699–707.

Hardwicke, J. T., Hart, J., Bell, A., Duncan, R., Thomas, D. W. and Moseley, R. (2011) The effect of dextrin-rhEGF on the healing of full-thickness, excisional wounds in the (db/db) diabetic mouse. *J Control Release*, **152,** 411–417.

Heller, J., Penhale, D.W.H., Helwing, R.F. (1980) Preparation of polyacetals by the reaction of divinyl ethers and polyols. *J Polym Sci Part C: Polym Lett*, **18**, 5–11.

Hoare, T., Santamaria, J., Goya, G. F., Irusta, S., Lin, D., Lau, S., Padera, R., Langer, R. and Kohane, D. S. (2009) A magnetically triggered composite membrane for on-demand drug delivery. *Nano Lett*, **9,** 3651–3657.

Hu, J., Zhang, G. and Liu, S. (2012) Enzyme-responsive polymeric assemblies, nanoparticles and hydrogels. *Chem Soc Rev*, **41,** 5933–5949.

Husseini, G. A. and Pitt, W. G. (2009) Ultrasonic-activated micellar drug delivery for cancer treatment. *J Pharm Sci*, **98,** 795–811.

Jung-Kwon, O. (2011) Polylactide (PLA)-based amphiphilic block copolymers: synthesis, self-assembly, and biomedical applications. *Soft Matter*, **7**, 5096–5108.

Kabanov, A. V., Batrakova, E. V. and Alakhov, V. Y. (2002) Pluronic® block copolymers as novel polymer therapeutics for drug and gene delivery. *J Control Release*, **82,** 189–212.

Kamaly, N., Xiao, Z., Valencia, P. M., Radovic-Moreno, A. F. and Farokhzad, O. C. (2012) Targeted polymeric therapeutic nanoparticles: Design, development and clinical translation. *Chem Soc Rev*, **41,** 2971–3010.

Katz, J. S. and Burdick, J. A. (2010) Light-responsive biomaterials: Development and applications. *Macromol Biosci*, **10,** 339–348.

Kim, D., Lee, E. S., Oh, K. T., Gao, Z. G. and Bae, Y. H. (2008) Doxorubicin-loaded polymeric micelle overcomes multidrug resistance of cancer by double-targeting folate receptor and early endosomal pH. *Small*, **4,** 2043–2050.

Kost, J., Leong, K. and Langer, R. (1988) Ultrasonically controlled polymeric drug delivery. *Makromolekulare Chemie Macromol Symp*, **19,** 275–285.

Kovář, L., Strohalm, J., Chytil, P., Mrkvan, T., Kovář, M., Hovorka, O., Ulbrich, K. and Rihová, B. (2007) The same drug but a different mechanism of action: Comparison of free doxorubicin with two different N-(2-hydroxypropyl)methacrylamide copolymer-bound doxorubicin conjugates in EL-4 cancer cell line. *Bioconjugate Chem*, **18,** 894–902.

Kovář, M., Kovář, L., Subr, V., Etrych, T., Ulbrich, K., Mrkvan, T., Loucka, J. and Rihová, B. (2004) HPMA copolymers containing doxorubicin bound by a proteolytically or hydrolytically cleavable bond: comparison of biological properties in vitro. *J Control Release*, **99,** 301–314.

Ku, T.-H., Chien, M.-P., Thompson, M. P., Sinkovits, R. S., Olson, N. H., Baker, T. S. and Gianneschi, N. C. (2011) Controlling and switching the morphology of micellar nanoparticles with enzymes. *J Am Chem Soc*, **133,** 8392–8395.

Kumar, C. S. S. R. and Mohammad, F. (2011) Magnetic nanomaterials for hyperthermia-based therapy and controlled drug delivery. *Adv Drug Deliv Rev*, **63,** 789–808.

Kumar, G. S. and Neckers, D. C. (1989) Photochemistry of azobenzene-containing polymers. *Chem Rev*, **89,** 1915–1925.

Kumari, A., Yadav, S. K. and Yadav, S. C. (2010) Biodegradable polymeric nanoparticles based drug delivery systems. *Colloid Surf B: Biointerfaces*, **75,** 1–18.

Kyriakides, T. R., Cheung, C. Y., Murthy, N., Bornstein, P., Stayton, P. S. and Hoffman, A. S. (2002) pH-Sensitive polymers that enhance intracellular drug delivery in vivo. *J Control Release*, **78,** 295–303.

Lammers, T., Hennink, W. E. and Storm, G. (2008) Tumour-targeted nanomedicines: Principles and practice. *Brit J Cancer*, **99,** 392–397.

Lammers, T., Kiessling, F., Hennink, W. E. and Storm, G. (2012) Drug targeting to tumors: Principles, pitfalls and (pre-) clinical progress. *J Control Release*, **161,** 175–187.

Lee, E. S., Gao, Z., Kim, D., Park, K., Kwon, I. C. and Bae, Y. H. (2008) Super pH-sensitive multifunctional polymeric micelle for tumor pHe specific TAT exposure and multidrug resistance. *J Control Release*, **129,** 228–236.

Lehner, R., Wang, X., Wolf, M. and Hunziker, P. (2012) Designing switchable nanosystems for medical application. *J Control Release*, **161,** 307–316.

Letchford, K. and Burt, H. (2007) A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. *Eur J Pharm Biopharm*, **65,** 259–269.

Liu, R., Fraylich, M. and Saunders, B. R. (2009) Thermoresponsive copolymers: From fundamental studies to applications. *Colloid Polym Sci*, **287,** 627–643.

Liu S. and Armes S. P. (2001). The facile one-pot synthesis of shell cross-linked micelles in aqueous solution at high solids. *J Am Chem Soc*, **123,** 9910–9911.

Loadman, P. M., Bibby, M. C., Double, J. A., AL-Shakhaa, W. M. and Duncan, R. (1999) Pharmacokinetics of PK1 and doxorubicin in experimental colon tumor models with differing responses to PK1. *Clin Cancer Res*, **5,** 3682–3688.

Maeda, H. (2012) Macromolecular therapeutics in cancer treatment: The EPR effect and beyond. *J Control Release*, **164**, 138–144..

Manchun, S., Dass, C. R. and Sriamornsak, P. (2012) Targeted therapy for cancer using pH-responsive nanocarrier systems. *Life Sci*, **90,** 381–387.

Mao, J. and Gan, Z. (2009) The influence of pendant hydroxyl groups on enzymatic degradation and drug delivery of amphiphilic poly[glycidol-block-(epsilon-caprolactone)] copolymers. *Macromol Biosci*, **9,** 1080–1089.

Marin, A., Muniruzzaman, M. and Rapoport, N. (2001) Mechanism of the ultrasonic activation of micellar drug delivery. *J Control Release*, **75,** 69–81.

Markovsky, E., Baabur-Cohen, H., Eldar-Boock, A., Omer, L., Tiram, G., Ferber, S., Ofek, P., Polyak, D., Scomparin, A. and Satchi-Fainaro, R. (2012) Administration, distribution, metabolism and elimination of polymer therapeutics. *J Control Release*, **161,** 446–460.

Marmottant, P. and Hilgenfeldt, S. (2003) Controlled vesicle deformation and lysis by single oscillating bubbles. *Nature*, **423,** 153–156.

Medintz, I. L., Uyeda, H. T., Goldman, E. R. and Mattoussi, H. (2005) Quantum dot bioconjugates for imaging, labelling and sensing. *Nat Mat*, **4,** 435–446.

Mellman, I. (1996) Endocytosis and molecular sorting. *Ann Rev Cell Dev Biol*, **12,** 575–625.

Meng, F., Hennink, W. E. and Zhong, Z. (2009) Reduction-sensitive polymers and bioconjugates for biomedical applications. *Biomaterials*, **30,** 2180–2198.

Minelli, C., Lowe, S. B. and Stevens, M. M. (2010) Engineering nanocomposite materials for cancer therapy. *Small*, **6,** 2336–2357.

Mort, J. S. and Buttle, D. J. (1997) Cathepsin B. *Int J Biochem Cell Biol*, **29,** 715–720.

Mrkvan, T., Sirova, M., Etrych, T., Chytil, P., Strohalm, J., Plocova, D., Ulbrich, K. and Rihová, B. (2005) Chemotherapy based on HPMA copolymer conjugates with pH-controlled release of doxorubicin triggers anti-tumor immunity. *J Control Release*, **110,** 119–129.

Munshi, N., Rapoport, N. and Pitt, W. G. (1997) Ultrasonic activated drug delivery from Pluronic P-105 micelles. *Cancer Lett*, **118,** 13–19.

Neuberger, T., Schöpf, B., Hofmann, H., Hofmann, M. and Von Rechenberg, B. (2005) Superparamagnetic nanoparticles for biomedical applications: Possibilities and limitations of a new drug delivery system. *J Magn Magn Mat*, **293,** 483–496.

Nie, Z., Petukhova, A. and Kumacheva, E. (2009) Properties and emerging applications of self-assembled structures made from inorganic nanoparticles. *Nat Nanotechnol*, **5,** 15–25.

Pankhurst, Q. A., Connolly, J., Jones, S. K. and Dobson, J. (2003) Applications of magnetic nanoparticles in biomedicine. *J Phys D: Appl Phys*, **36,** R167–R181.

Parveen, S. and Sahoo, S. K. (2008) Polymeric nanoparticles for cancer therapy. *J Drug Target*, **16,** 108–123.

Peer, D., Karp, J. M., Hong, S., Farokhzad, O. C., Margalit, R. and Langer, R. (2007) Nanocarriers as an emerging platform for cancer therapy. *Nat Nanotechnol*, **2,** 751–760.

Richardson, S. C. W., Pattrick, N. G., Lavignac, N., Ferruti, P. and Duncan, R. (2010) Intracellular fate of bioresponsive poly(amidoamine)s in vitro and in vivo. *J Control Release*, **142,** 78–88.

Rijcken, C. J., Snel, C. J., Schiffelers, R. M., van Nostrum, C. F. and Hennink, W. E. (2007) Hydrolysable core-crosslinked thermosensitive polymeric micelles: Synthesis, characterisation and in vivo studies. *Biomaterials*, **28,** 5581–5593.

Rijcken, C. J. F., Talelli, M., van Nostrum, C. F., Storm, G. and Hennink, W. E. (2010) Crosslinked micelles with transiently linked drugs – A versatile drug delivery system. *Eur J Nanomed*, **3,** 19–24.

Rodrigues, P. C. A., Beyer, U., Schumacher, P., Roth, T., Fiebig, H. H., Unger, C., Messori, L., Orioli, P., Paper, D. H., Milhaupt, R. and Kratz, F. (1999) Acid-sensitive polyethylene glycol conjugates of doxorubicin: preparation, in vitro efficacy and intracellular distribution. *Bioorg Med Chem*, **7,** 2517–2524.

Rodrigues, P. C. A., Roth, T., Fiebig, H. H., Unger, C., Milhaupt, R. and Kratz, F. (2006) Correlation of the acid-sensitivity of polyethylene glycol daunorubicin conjugates with their in vitro antiproliferative activity. *Bioorg Med Chem*, **14,** 4110–4117.

Roy, D., Cambre, J. N. and Sumerlin, B. S. (2010) Future perspectives and recent advances in stimuli-responsive materials. *Prog Polym Sci*, **35,** 278–301.

Sanchis, J., Canal, F., Lucas, R. and Vicent, M. J. (2010) Polymer-drug conjugates for novel molecular targets. *Nanomedicine*, **5,** 915–935.

Santamaria, B., Benito-Martin, A., Conrado Ucero, A., Stark Aroeira, L., Reyero, A., Jesus Vicent, M., Orzaez, M., Celdran, A., Esteban, J., Selgas, R., Ruiz-Ortega, M., Lopez Cabrera, M., Egido, J., Perez-Paya, E. and Ortiz, A. (2009) A Nanoconjugate Apaf-1 inhibitor protects mesothelial cells from cytokine-induced injury. *Plos One*, **4**, e6634.

Schmaljohann, D. (2006) Thermo- and pH-responsive polymers in drug delivery. *AdvDrug Deliv Rev*, **58,** 1655–1670.

Segal, E., Pan, H., Benayoun, L., Kopeckova, P., Shaked, Y., Kopecek, J. and Satchi-Fainaro, R. (2011) Enhanced anti-tumor activity and safety profile of targeted

nano-scaled HPMA copolymer-alendronate-TNP-470 conjugate in the treatment of bone malignancies. *Biomaterials*, **32,** 4450–4463.

Segal, E., Pan, H., Ofek, P., Udagawa, T., Kopeckova, P., Kopecek, J. and Satchi-Fainaro, R. (2009) Targeting angiogenesis-dependent calcified neoplasms using combined polymer therapeutics. *Plos One*, **4**, e5233.

Seymour, L. W., Ferry, D. R., Anderson, D., Hesslewood, S., Julyan, P. J., Poyner, R., Doran, J., Young, A. M., Burtles, S. and Kerr, D. J. (2002) Hepatic drug targeting: Phase I evaluation of polymer-bound doxorubicin. *J Clin Oncol*, **20,** 1668–1676.

Seymour, L. W., Ferry, D. R., Kerr, D. J., Rea, D., Whitlock, M., Poyner, R., Boivin, C., Hesselewood, S., Twelves, C., Blackie, R., Schatzlein, A., Jodrell, D., Bissett, D., Calvert, H., Lind, M., Robbins, A., Burtles, S., Duncan, R. and Cassidy, J. (2009) Phase II studies of polymer-doxorubicin (PK1, FCE28068) in the treatment of breast, lung and colorectal cancer. *Int J Oncol*, **34,** 1629–1636.

Shaunak, S., Thomas, S., Gianasi, E., Godwin, A., Jones, E., Teo, I., Mireskandari, K., Luthert, P., Duncan, R., Patterson, S., Khaw, P. and Brocchini, S. (2004) Polyvalent dendrimer glucosamine conjugates prevent scar tissue formation. *Nat Biotechnol*, **22,** 977–984.

Shenoy, D., Little, S., Langer, R. and Amiji, M. (2005) Poly(ethylene oxide)-modified poly(Ξ²-amino ester) nanoparticles as a pH-sensitive system for tumor-targeted delivery of hydrophobic drugs: Part 2. In vivo distribution and tumor localization studies. *Pharm Res*, **22,** 2107–2114.

Singer, J. W., Bhatt, R., Tulinsky, J., Buhler, K. R., Heasley, E., Klein, P. and de Vries, P. (2001) Water-soluble poly-(L-glutamic acid)-Gly-camptothecin conjugates enhance camptothecin stability and efficacy in vivo. *J Control Release*, **74,** 243–247.

Singer, J. W., de Vries, P., Bhatt, R., Tulinsky, J., Klein, P., Li, C., Milas, L., Lewis, R. A. and Wallace, S. (2000) Conjugation of camptothecins to poly(L-glutamic acid). *Ann NY Acad Sci*, **922**, 136–150.

Sirova, M., Mrkvan, T., Etrych, T., Chytil, P., Rossmann, P., Ibrahimova, M., Kovář, L., Ulbrich, K. and Rihová, B. (2010) Preclinical evaluation of linear HPMA-doxorubicin conjugates with pH-sensitive drug release: efficacy, safety, and immunomodulating activity in murine model. *Pharm Res*, **27,** 200–8.

Soga, O., van Nostrum, C. F., Fens, M., Rijcken, C. J. F., Schiffelers, R. M., Storm, G. and Hennink, W. E. (2005a) Thermosensitive and biodegradable polymeric micelles for paclitaxel delivery. *J Control Release*, **103,** 341–353.

Soga, O., van Nostrum, C. F. and Hennink, W. E. (2004a) Poly(N-(2-hydroxypropyl) methacrylamide mono/di lactate): A new class of biodegradable polymers with tuneable thermosensitivity. *Biomacromolecules*, **5,** 818–821.

Soga, O., van Nostrum, C. F. and Hennink, W. E. (2005b) Thermosensitive and biodegradable polymeric micelles with transient stability. *J Control Release*, **101,** 383–385.

Soga, O., van Nostrum, C. F., Ramzi, A., Visser, T., Soulimani, F., Frederik, P. M., Bomans, P. H. H. and Hennink, W. E. (2004b) Physicochemical characterization of degradable thermosensitive polymeric micelles. *Langmuir*, **20,** 9388–9395.

Song, N., Liu, W., Tu, Q., Liu, R., Zhang, Y. and Wang, J. (2011) Preparation and in vitro properties of redox-responsive polymeric nanoparticles for paclitaxel delivery. *Colloid Surf B: Biointerfaces*, **87,** 454–463.

Stevens, M. M., Flynn, N. T., Wang, C., Tirrell, D. A. and Langer, R. (2004) Coiled-coil peptide-based assembly of gold nanoparticles. *Adv Mat*, **16,** 915–918.

Sun, P., Zhou, D. and Gan, Z. (2011) Novel reduction-sensitive micelles for triggered intracellular drug release. *J Control Release*, **155,** 96–103.

Talelli, M. and Hennink, W. E. (2011) Thermosensitive polymeric micelles for targeted drug delivery. *Nanomedicine*, **6,** 1245–1255.

Talelli, M., Iman, M., Varkouhi, A. K., Rijcken, C. J., Schiffelers, R. M., Etrych, T., Ulbrich, K., van Nostrum, C. F., Lammers, T., Storm, G. and Hennink, W. E. (2010a) Core-crosslinked polymeric micelles with controlled release of covalently entrapped doxorubicin. *Biomaterials*, **31,** 7797–804.

Talelli, M., Iman, M., Varkouhi, A. K., Rijcken, C. J. F., Schiffelers, R. M., Etrych, T., Ulbrich, K., van Nostrum, C. F., Lammers, T., Storm, G. and Hennink, W. E. (2010b) Core-crosslinked polymeric micelles with controlled release of covalently entrapped doxorubicin. *Biomaterials*, **31,** 7797–7804.

Talelli, M., Morita, K., Rijcken, C. J. F., Aben, R. W. M., Lammers, T., Scheeren, H. W., van Nostrum, C. F., Storm, G. and Hennink, W. E. (2011) Synthesis and characterization of biodegradable and thermosensitive polymeric micelles with covalently bound doxorubicin-glucuronide prodrug via click chemistry. *Bioconjugate Chem*, **22,** 2519–2530.

Tang, H., Murphy, C. J., Zhang, B., Shen, Y., van Kirk, E. A., Murdoch, W. J. and Radosz, M. (2010) Curcumin polymers as anticancer conjugates. *Biomaterials*, **31,** 7139–7149.

Tomlinson, R., Heller, J., Brocchini, S. and Duncan, R. (2003) Polyacetal–doxorubicin conjugates designed for pH-dependent degradation. *Bioconjugate Chem*, **14,** 1096–1106.

Tomlinson, R., Klee, M., Garrett, S., Heller, J., Duncan, R. and Brocchini, S. (2002) Pendent chain functionalized polyacetals that display pH-dependent degradation: A platform for the development of novel polymer therapeutics. *Macromolecules*, **35,** 473–480.

Tong, X., Wang, G., Soldera, A. and Zhao, Y. (2005) How can azobenzene block copolymer vesicles be dissociated and reformed by light? *J Phys Chem B*, **109,** 20281–20287.

Torchilin, V. P. (2000) Drug targeting. *Eur J Pharm Sci*, **11**, Suppl 2 S81–S91.

Tyagarajan, K., Pretzer, E. and Wiktorowicz, J. E. (2003) Thiol-reactive dyes for fluorescence labeling of proteomic samples. *Electrophoresis*, **24,** 2348–2358.

Ulbrich, K., Etrych, T., Chytil, P., Jelínková, M. and Říhová, B. (2003) HPMA copolymers with pH-controlled release of doxorubicin: In vitro cytotoxicity and in vivo antitumor activity. *J Control Release*, **87,** 33–47.

Valle, J. W., Armstrong, A., Newman, C., Alakhov, V., Pietrzynski, G., Brewer, J., Campbell, S., Corrie, P., Rowinsky, E. K. and Ranson, M. (2011) A phase 2 study of SP1049c, doxorubicin in P-glycoprotein-targeting pluronics, in patients with advanced adenocarcinoma of the esophagus and gastroesophageal junction. *Invest New Drug*, **29,** 1029–1037.

Vasey, P. A., Kaye, S. B., Morrison, R., Twelves, C., Wilson, P., Duncan, R., Thomson, A. H., Murray, L. S., Hilditch, T. E., Murray, T., Burtles, S., Fraier, D., Frigerio, E. and Cassidy, J. (1999a) Phase I clinical and pharmacokinetic study of PK1 [N-(2-hydroxypropyl)methacrylamide copolymer doxorubicin]: First member of a

new class of chemotherapeutic agents – Drug-polymer conjugates. *Clin Cancer Res*, **5,** 83–94.

Vicent, M.J., Greco, F., Paul, A., Griffiths, P.C., Nicholson, R.I., Duncan, R. (2005) Polymer Therapeutics Designed for a Combination Therapy of Hormone-Dependent Cancer. *Angew Chem Int Ed*, **44**, 2–6.

Vicent, M. J., Ringsdorf, H. and Duncan, R. (2009) Polymer therapeutics: Clinical applications and challenges for development. *AdvDrug Deliv Rev*, **61,** 1117–1120.

Vicent, M. J., Tomlinson, R., Brocchini, S. and Duncan, R. (2004) Polyacetaldiethylstilboestrol: A polymeric drug designed for ph-triggered activation. *J Drug Target*, **12,** 491–501.

Wang, G., Tong, X. and Zhao, Y. (2004) Preparation of azobenzene-containing amphiphilic diblock copolymers for light-responsive micellar aggregates. *Macromolecules*, **37,** 8911–8917.

Wang, Y. C., Wang, F., Sun, T. M. and Wang, J. (2011) Redox-responsive nanoparticles from the single disulfide bond-bridged block copolymer as drug carriers for overcoming multidrug resistance in cancer cells. *Bioconjugate Chem*, **22,** 1939–1945.

Wang, Y. X. J., Hussain, S. M. and Krestin, G. P. (2001) Superparamagnetic iron oxide contrast agents: Physicochemical characteristics and applications in MR imaging. *Eur Radiol*, **11,** 2319–2331.

Warnecke, A., Fichtner, I., Sass, G. and Kratz, F. (2007) Synthesis, cleavage profile, and antitumor efficacy of an albumin-binding prodrug of methotrexate that is cleaved by plasmin and cathepsin B. *Archiv Der Pharmazie*, **340,** 389–395.

Wei, H., Cheng, S. X., Zhang, X. Z. and Zhuo, R. X. (2009) Thermo-sensitive polymeric micelles based on poly(N-isopropylacrylamide) as drug carriers. *Prog Polym Sci (Oxford)*, **34,** 893–910.

Wu, D., Wang, H., Li, Z., Wang, L., Zheng, F., Jiang, J., Gao, Y., Zhong, H., Huang, Y. and Suo, Z. (2012) Cathepsin B may be a potential biomarker in cervical cancer. *Histol Histopathol*, **27,** 79–87.

Wu, G., Fang, Y. Z., Yang, S., Lupton, J. R. and Turner, N. D. (2004) Glutathione metabolism and its implications for health. *J Nutrit*, **134,** 489–492.

Yan, Y., Johnston, A. P. R., Dodds, S. J., Kamphuis, M. M. J., Ferguson, C., Parton, R. G., Nice, E. C., Heath, J. K. and Caruso, F. (2010) Uptake and intracellular fate of disulfide-bonded polymer hydrogel capsules for doxorubicin delivery to colorectal cancer cells. *ACS Nano*, **4,** 2928–2936.

Yigit, M. V., Moore, A. and Medarova, Z. (2012) Magnetic nanoparticles for cancer diagnosis and therapy. *Pharm Res*, **29,** 1180–1188.

Zelzer, M. and Ulijn, R. V. (2010) Next-generation peptide nanomaterials: molecular networks, interfaces and supramolecular functionality. *Chem Soc Rev*, **39,** 3351–3357.

Zhang, H., Fu, T., Mcgettigan, S., Kumar, S., Liu, S., Speicher, D., Schuchter, L. and Xu, X. (2011a) IL8 and cathepsin B as melanoma serum biomarkers. *Int J Mol Sci*, **12,** 1505–1518.

Zhang, H., Li, F., Yi, J., Gu, C., Fan, L., Qiao, Y., Tao, Y., Cheng, C. and Wu, H. (2011b) Folate-decorated maleilated pullulan–doxorubicin conjugate for active tumor-targeted drug delivery. *Eur J PharmSci*, **42,** 517–526.

Zhao, H., Sterner, E. S., Coughlin, E. B. and Theato, P. (2012) O-Nitrobenzyl alcohol derivatives: Opportunities in polymer and materials science. *Macromolecules*, **45,** 1723–1736.

Zhao, M., Biswas, A., Hu, B., Joo, K. I., Wang, P., Gu, Z. and Tang, Y. (2011) Redox-responsive nanocapsules for intracellular protein delivery. *Biomaterials*, **32,** 5223–5230.

Zhao, Y. (2012) Light-responsive block copolymer micelles. *Macromolecules*, **45,** 3647–3657.

12

The use of smart polymers in medical devices for minimally invasive surgery, diagnosis and other applications

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Abstract: This chapter describes the types of smart polymers, the technologies used for their production and their applications in the field of medical devices. Stimuli-responsive polymers are classified according to their behaviour in response to the environment and on the basis of their structural properties. Most advanced strategies for the design of intelligent macromolecular systems are also reviewed, with an emphasis on the nanopatterning of surfaces. The main applications of these materials for the design of novel medical devices, which include minimally invasive surgery, cancer diagnosis and therapy, biosensors, bioactuators and microfluidics-based systems, are discussed.

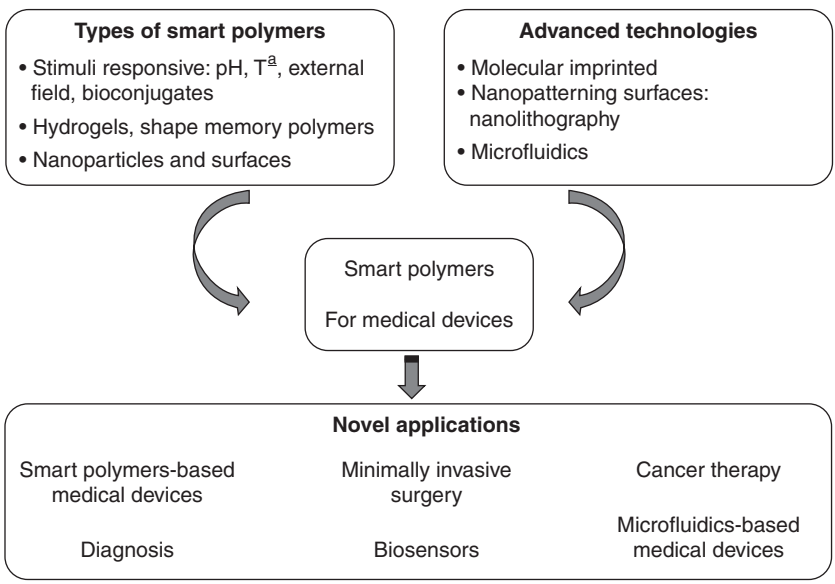
Key words: smart medical devices, minimally invasive surgery, microfluidics and biosensors.

12.1 Introduction

Medical devices have been described as instruments, apparatus, machines, implants, *in vitro* reagents or other accessories designed for use in the diagnosis or treatment of diseases without the need for chemical action or metabolism in the human body (http://www.fda.gov/MedicalDevices/). Throughout history, stimuli-responsive polymers have played an important role in the treatment of different diseases and the improvement of health care of patients. Response to stimuli is an elemental process in nature and living systems. The maintenance of living cells is also regulated by macromolecules that respond to changes in the local environment. These examples have inspired the fabrication of smart polymers that respond to stimuli such as temperature, pH, light or ionic strength by changes in their shape, solubility, surface properties, volume, etc.

Smart or stimuli-responsive polymers have become very attractive materials for biomedical applications because of the possibility of controlling their properties for specific uses in fields such as minimally invasive surgery (MIS), the development of implants, biosensors, bioactuactors and artificial muscles as well as *in vitro* diagnostics, arrays and microfluidics-based systems with high biocompatibility, sensitivity and efficiency (Jeong and Gutowska, 2002; Kirsebom *et al.*, 2010; Roy and Gupta, 2003; Roy *et al.*, 2010). Recent advances in the design of stimuli-responsive polymers have enabled the creation of new opportunities for biomedical applications, including the development and fabrication of more advanced medical devices (Anderson *et al.*, 2004; Langer and Tirrell, 2004).

Advances in medical treatments for a wide range of diseases require the development of highly sensitive and efficient systems and approaches through the use of non-toxic, biocompatible and biodegradable polymers. Currently, knowledge of nanotechnology and the design of materials with different structural properties are making possible new routes to fight a number of diseases by providing stimuli-responsive structures capable of undergoing conformational and chemical changes in response to an external signal. Moreover, the combination of micro- and nanofabricated systems with smart polymers is an exciting route for the development of better diagnostic and therapeutic medical devices (Cabane *et al.*, 2012; Caldorera-Moore and Peppas, 2009; Stuart *et al.*, 2010). This chapter focuses on the



12.1 Summary of contents.

description of the most advanced types and technologies for the design of intelligent polymers and their novel applications in the field of medical devices, including examples to illustrate particular applications (see Fig. 12.1).

12.2 Types and preparation of smart polymers for medical devices: polymers classified by type of stimulus

There has been interest in smart polymers for many decades. Currently, a great deal of effort is being dedicated to developing environmentally sensitive polymers for the fabrication of new smart materials for use in the most advanced and sophisticated medical devices (Ravichandran *et al.*, 2012; Roy *et al.*, 2010). This section and the following one deal with the most relevant types of smart polymers in terms of stimuli-responsive behaviour and structural properties, including new technologies for the synthesis of macromolecules that are able to form highly diversified structures.

Physical and chemical stimuli such as temperature and pH are the most common stimuli used in the design of smart polymers. Other possible external stimuli, such as electric fields, have also been considered in the development of these systems. Finally, biological stimuli, such as the presence of specific biomolecules, are also able to change the properties of intelligent macromolecules (Cabane *et al.*, 2012; Chan *et al.*, 2012; Ravichandran *et al.*, 2012; Roy *et al.*, 2010). Advanced studies on different stimuli-responsive polymers are described below.

12.2.1 Physically dependent stimuli: temperature-responsive polymers

Physically dependent stimuli include a wide range of variables such as temperature and mechanical deformation, amongst others. However, thermoresponsive polymers are the most relevant class of smart polymers studied because of their enormous variety and their great potential for different biomedical applications. Usually these polymers have both hydrophilic and hydrophobic phases, and undergo abrupt changes in their electrostatic and hydrophobic interactions in an aqueous medium at a critical solution temperature.

Varying in the mechanism and chemistry of the functional groups different temperature-responsive polymers have been reported in recent years. The most common behaviour of these materials is characterized by polymer solutions that appear monophasic below a specific lower critical solution temperature (LCST), and biphasic above it. Some interesting examples are

poly(N-alkyl substituted acrylamides), poly(N-vinylalkylamides), poly(N-vinyl piperidine) and poly(N-vinylcaprolactam) (Cabane *et al.*, 2012; Chan *et al.*, 2012; Ravichandran *et al.*, 2012).

Poly(N-isopropylacrylamide) (PNIPAM) is the most representative thermo-responsive polymer. It has an abrupt transition temperature at approximately 32°C, with an extensive number of applications reported in the literature (Chan *et al.*, 2012; Hoffman, 1987; Jeong and Gutowska, 2002; Ravichandran *et al.*, 2012). The importance of this intelligent polymer in the field of biomedical applications is due to the closeness of its LCST to human body temperature, together with the possibility of changing this temperature by copolymerization with other appropriate monomers. For that reason, PNIPAM has been used as a diagnostic reagent for assay technologies and for the detection of biomarkers, enhancing the efficacy of immunodiagnostic systems by separating and concentrating sample analytes through thermal aggregation and phase separation above its LCST (Nash *et al.*, 2010). Another interesting application of this polymer is for cancer treatment by hyperthermia (Purushotham and Ramanujan, 2010 ; Wust, 2002). In all cases, the development of medical applications is possible because of their adsorption onto the surfaces of microfluidic devices or functionalized nanoparticles (NPs) (Nash *et al.*, 2010).

However, the development of *in vivo* applications for PNIPAM is limited by its non-biodegradability and the presence of amide moieties that reduce its biocompatibility. For this reason, other thermo-responsive polymers have been investigated in recent years. Poly(N-vinylcaprolactam) is a promising alternative. This polymer has a LCST between 35 and 38°C, again close to the temperature of the human body, and is characterized by high biocompatibility and low toxicity (Koňák *et al.*, 2007; Medeiros *et al.*, 2010; Shtanko *et al.*, 2003; Yanul *et al.*, 2001). Additionally, amphiphilic copolymers such as Pluronics® and Tetronics® have been developed, based on copolymers of polyethylene oxide and polypropylene oxide. These copolymer systems exhibit a solution–gel transition at close to human body temperature that permits their application as injectable implants (Samchenko *et al.*, 2011).

12.2.2 Chemically dependent stimuli: pH-responsive polymers

Chemical stimuli typically comprise pH, solvent, redox and ionic strength. In the field of medical devices, pH-responsive polymers have been used successfully because different cellular compartments, tissues and organs in the human body undergo variations in pH. For example, in the gastrointestinal tract, pH changes from values between 1 and 3 in the stomach to levels higher than 6 in the intestines.

pH-responsive polymers are based on the presence of ionizable weak acidic (carboxylic acid, phosphoric acid) or basic (amines, ammonia) groups linked to the polymer structure. These moieties are able to accept or release protons in response to changes in environmental pH, which produces changes in the solubility and in the swelling properties of the polymers. Typical pH-responsive polymers are poly(acrylic acid), poly(methacrylic acid)s, poly(vinylpyridine), chitosan and gelatin, amongst others (Cabane *et al.*, 2012; Chan *et al.*, 2012; Ravichandran *et al.*, 2012). These materials have enhanced the development of biosensors and, in particular, sensors for monitoring blood glucose levels (Liu *et al.*, 1997; Podual *et al.*, 2000; Roy *et al.*, 2010; Tanna, 2006; Wang, 2001; Zhao *et al.*, 2011).

12.2.3 Field-responsive polymers: electro- and photo-responsive polymers

Electro-responsive polymers are materials that can regulate their properties, such as swelling, shrinkage and bending, in response to an electric field. Moreover, electro-responsive polymers can transform electrical energy into accurately-controlled mechanical energy through regulation of the current, the duration of the electrical pulse or the interval between pulses. These properties have been used in the fabrication of artificial muscles, actuators and biosensors that allow improvements in advanced miniature biomedical and microfluidic systems for point-of-care (POC) devices (Cabane *et al.*, 2012; Ravichandran *et al.*, 2012; Roy *et al.*, 2010). The most typical materials investigated in this field are polyelectrolyte hydrogels based on both natural and synthetic polymers, such as hyaluronic acid, chitosan, poly(vinyl alcohol), poly(acrylic acid) and poly(methacrylic acid). The advantage of polyelectrolyte hydrogels is that they have a directional response due to anisotropic swelling or shrinkage in an electric field (Filipcsei *et al.*, 2000; Gao *et al.*, 2008; Kim *et al.*, 2004).

Light is another attractive source of energy for the development of intelligent biomaterials, which can be designed to switch their properties when irradiated by light of the appropriate wavelength. Moreover, the wavelength and intensity can be controlled using filters, photomasks or lasers to permit complex features with high resolution. Photo-responsive polymers have been investigated in applications such as photomechanical actuation and bioactivity switching of proteins. The use of light as a stimulus is particularly attractive in the field of medical devices, because the mechanism to induce the response is non-invasive, being minimally absorbed by tissue or organs, and maximally by the material. Typically, photo-responsive polymers are characterized by the presence of photoactive groups such as azobenzene, spirobenzopyran, triphenylmethane or cinnamonyl along the

polymer backbone, or by side chains that are able to undergo reversible structural changes under light irradiation. These photoactive groups have been incorporated into a wide variety of polymers such as poly(acrylic acid) or PNIPAM (Cabane, 2012; Katz and Burdick, 2010; Ravichandran *et al.*, 2012; Roy *et al.*, 2010; Shimoboji *et al.*, 2002).

12.2.4 Biologically dependent stimuli: bioconjugates

Nowadays, there is increasing interest in the development of switchable polymers sensitive to specific chemical analytes or biomolecules, such as proteins, glucose and DNA. Recent advances in the fields of biotechnology and nanotechnology have significantly focused attention on the combination of polymers of both natural and synthetic origin, with biomolecules generally referred to as 'polymer bioconjugates'. This modern strategy allows the preparation of hybrid polymers with excellent properties, combining the complexity and functionality of biological systems with the possibility of structural chemical design. However, the development of these materials is not recent; the first paper on the preparation of conjugates of poly(ethylene glycol) (PEG) with protein drugs was published in 1977. These conjugates prevent the immune system from recognizing the protein in the body. In the early 1980s, Hoffman and coworkers linked temperature-responsive polymers to proteins (Hoffman and Stayton, 2004; Hoffman and Stayton, 2007; Hoffman *et al.*, 2000).

A wide variety of biomacromolecules have been investigated for the preparation of bioconjugates, including proteins, polysaccharides, DNA plasmids, lipids and phospholipids. However, the most attractive and versatile systems are based on the conjugation of thermo-responsive polymers to different proteins, useful for a great variety of medical devices such as implants, diagnostics, biosensors and immunoassays (Gupta and Mattiasson, 2006; Hoffman, 2000; Pennadam, 2004).

The preparation of polymer–protein conjugates is possible by two mechanisms: random and site-specific conjugation. In the case of random conjugation, the polymer is usually linked to lysine groups of the proteins. Site-specific conjugation, on the other hand, is based on the insertion of cysteine residues with exposed thiol groups which react preferentially with vinyl or vinyl sulfon groups of the smart polymers (Hoffman and Stayton, 2007).

Smart polymer–streptavidin conjugates have been intensively studied by Hoffman and coworkers. An example is the preparation of copolymers based on acrylic acid and N-isopropylacrylamide (NIPAM) with high sensitivity to both pH and temperature, in a useful range of these properties. This copolymer is conjugated to a specific cysteine thiol site inserted by genetic

engineering into the recognition site of streptavidin. This design allows pH control of biotin binding and triggers release of a genetically modified protein. The effect of pH and temperature on the triggered-release of biotin is useful in many diagnostic applications and medical devices, particularly, for example, where the pH is significantly below 7, such as the stomach, the vagina, the salivary glands and within intracellular vesicles. However, *in vivo* applications of these systems present some limitations because bioconjugates need to be eliminated from the body within a reasonable time after administration. For that reason, exhaustive control of their molecular weight is desirable in the development of medical devices based on bioconjugates. Currently, controlled radical polymerization, ring opening polymerization or click chemistry have been used as extremely versatile tools for the preparation of tailor-made polymer bioconjugates. The effective implementation of these polymerization techniques in the synthesis of bioconjugates will be a critical factor in the advancement of applications for these polymeric systems (Bulmus *et al.*, 1999; Lutz and Börner, 2008).

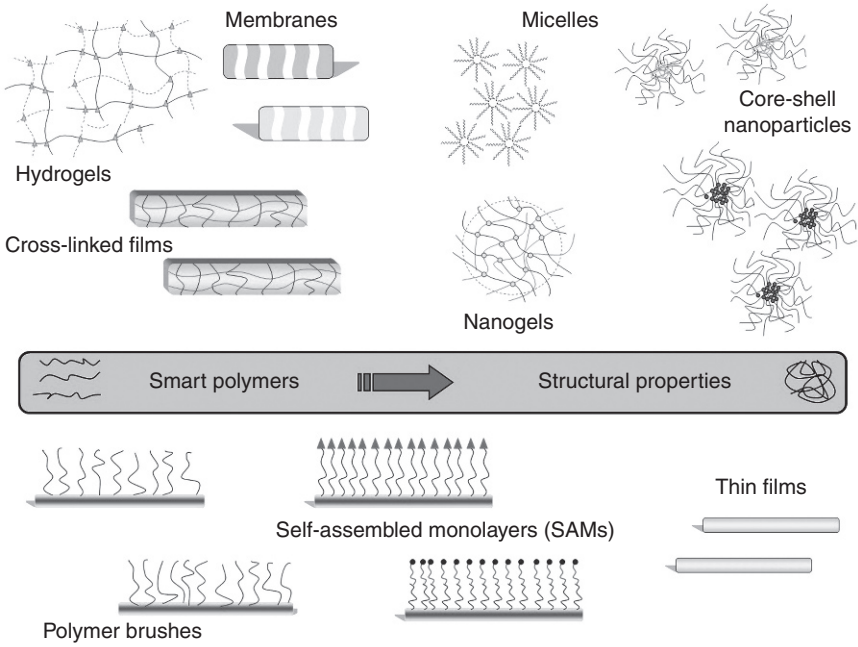
12.3 Types and preparation of smart polymers for medical devices: polymers classified by structural properties

Stimuli-responsive systems have been designed for different architectures and specific structural properties, and have enhanced the development of new biosensors and actuators, microfluidic devices, diagnostic systems and new therapeutic treatments for diseases. Intelligent surfaces, NPs, gels and shape memory polymers (SMPs) are considered below (see Fig. 12.2).

12.3.1 Smart hydrogels

Novel approaches and technologies in the design of hydrogels have led to the development of a wide variety of materials with different structures and properties with potential applications in medical devices. Some remarkable examples are hybrid and double network hydrogels, sliding cross-linking agents, nanocomposite hydrogels and superporous gels (Chaterji *et al.*, 2007; Kopeček, 2007; Jagur-Grodzinski, 2010).

pH- and temperature-responsive hydrogels are the most widely studied responsive hydrogel systems that have been used in biosensors and actuators, as they can be integrated into microdevices using nanopatterning technologies such as nanolithography. Also, analyte-sensitive gels are an excellent tool in the field of medical diagnosis because they are able to determine the concentrations of various biologically relevant substances (Deligkaris *et al.*, 2010; Jagur-Grodzinski, 2010; Kopeček, 2007; Kopeček and Yang, 2007).



12.2 Classification of smart polymers on the basis of their structural properties.

Analyte-sensitive hydrogels can be designed to test swelling changes in response to an increase in the concentration of specific biomolecules such as glucose, proteins or peptides. A practical example is the widely researched glucose-sensitive hydrogels that are able to sense the levels of blood glucose and release insulin through the incorporation of immobilized enzymes, specifically glucose oxidase (GOx), into pH-sensitive cationic hydrogels (Farmer *et al.*, 2008; Hoare and Pelton, 2008; Kang and Bae, 2003; Lapeyre *et al.*, 2008; Podual *et al.*, 2000; Zhang *et al.*, 2008). Another interesting possibility is the preparation of antigen-sensitive hydrogels which can be used to make a wide variety of sensing devices, particularly in immunoassay for the detection and measurement of biological and non-biological substances. In these cases, hydrogels are prepared by physically entrapping antibodies or antigens in networks, by their chemical conjugation to the network, or by using antigen–antibody pairs as reversible cross-linkers (Chaterji *et al.*, 2007; Roy *et al.*, 2010). Miyata *et al.* (1999a, b) developed antigen–antibody-responsive gels using rabbit immunoglobulin G as an antigen. Hydrogels from polymerizable antibody Fab' fragments and membranes based on a cross-linked dextran backbone grafted with a fluorescein isothiocyanate antigen are other examples of these responsive systems (Lu *et al.*, 2003; Zhang *et al.*, 2007b).

Currently, the improvement and implantation of analyte-responsive gels for medical devices has been possible due to the expansion of molecular imprinting technology. This emerging approach involves the formation of specific binding sites within the flexible structure of a hydrogel in order to recognize a wide variety of analytes with high affinity. It is achieved by polymerization with a certain degree of cross-linking with a functional monomer having a specific chemical structure designed to interact with the imprinted molecule (template). After polymerization, removal of the imprinted molecule from the polymer leaves a cavity with recognition sites that fit similar molecules (Byrne *et al.*, 2002).

Molecular imprinted gels with imprinted biomolecules such as glucose, cholesterol, lysozymes, DNA, peptides, proteins and low molecular weight compounds have been reported for medical devices (Chen *et al.*, 2008; Fu *et al.*, 2008; Hua *et al.*, 2008; Takeuchi and Hishiya, 2008; Wang *et al.*, 2008). One attractive example is the preparation of tumour marker-responsive gels. Miyata *et al.* reported the use of imprinted gels that exhibit volume changes in response to a tumour-specific marker glycoprotein (α-fetoprotein, AFP) using lectin and other antibody molecules as ligands. AFP imprinted gels can be used in molecular diagnostic methods because this glycoprotein shows an anomalous glycosylation process in various diseased states, such as primary hepatoma and cirrhosis of the liver (Ogiso *et al.*, 2006).

The use of molecular imprinted gels is particularly interesting for biosensors due to the possibility of avoiding some of the problems that prevent the marketing of these systems, such as unpredictable stability, low reproducibility and difficulties in incorporating biomolecules into sensor platforms. These systems offer the possibility of developing implantable microdevices, thus increasing the ability to sense early disease states (Hillberg *et al.*, 2005). Imprinted glucose sensors have been extensively investigated, with excellent results (Cheng *et al.*, 2001; Seong *et al.*, 2002). Another therapeutic sensing application is in the recognition of vanillylmandelic acid, which can be indicative of neuroblastoma disease when it is excreted at above-normal levels (Blanco-López *et al.*, 2003).

The emergence of nanotechnology has allowed the development of hydrogel thin films and membranes. Hydrogel thin films offer important advantages over grafted polymers in the fabrication of multifunctional and miniaturized devices with fast response times and high stability, due to the multiple anchoring points of the thin films to the surface. Hydrogel thin films can be used in the creation of novel 'lab-on-a-chip' (LOC) devices, where they are incorporated inside microfluidic channels or microvalves to operate as actuactors, based on their swelling ability. In this context, it is important to note that the swelling response of these films is highly anisotropic, and as a result, volumetric expansion of the network is possible only in the direction normal to the substrate plane. In addition, recent studies

have used hydrogel thin films for the immobilization of bioreceptors, such as DNA or proteins, and for monitoring biomolecular binding events (Tokarev and Minko, 2009b).

In the case of membranes, the most important advantage is the possibility of using stimuli-responsive control of the pores via the swelling processes to regulate the permeability of the membrane. The association between the changes in volume and pore size of intelligent membranes permits the development of a broad range of properties for use with specific chemicals, biomolecules or NPs (Stuart *et al.*, 2010; Tokarev and Minko, 2009a; Tokarev *et al.*, 2006; Wandera *et al.*, 2010). An interesting example of these particular systems is the promising use of gel membranes based on poly(2-vinylpyridine) through the cross-linking reaction of pyridine rings with 1,4-diiodobutane to form a polymer network. These membranes can operate as pH-controlled porous systems, when coated onto different supports. Moreover, biomolecules or NPs can be included inside their structure.

Recent studies have prepared poly(2-vinylpyridine) membranes on an electroconductive support, through the binding of cholesterol to monomer units in the membrane with the formation of strong hydrogen bonds. Complete characterization of the swelling behaviour of this membrane confirmed that the pore size depends on cholesterol concentration. This system can operate as an electrochemical gate, with applications for the development of biosensors. Recently, Tokarev and coworkers developed novel multiresponsive gel membranes from alginate with excellent biocompatibility (Tokarev and Minko, 2009a; Tokarev *et al.*, 2007, 2008).

12.3.2 Shape memory polymers (SMPs)

SMPs are very important smart polymers that are able to return to their original shape after being severely deformed under relevant stimuli such as temperature, light, electric field, magnetic field or pH (Behl *et al.*, 2010; Ravichandran *et al.*, 2012; Sun *et al.*, 2012). These materials are of low density and have reduced fabrication cost, whilst having good chemical stability and biocompatibility as well as recoverable strain in order of 100% (Sun *et al.*, 2012). For these reasons, SMPs are good candidates for stents, artificial muscles, orthodontics and MIS. The shape memory effect permits the shrinkage of surgical devices to a much smaller size. Moreover, shape memory stents are very attractive devices because they prevent tissue in-growth and infection and may reduce the risk of thrombosis associated with polymeric drug-eluting stents (Huang *et al.*, 2012; Takashima *et al.*, 2010).

Shape memory polyurethanes are one of the main classes of SMPs studied, particularly those activated by temperature (Hyun Kim *et al.*, 2010; Small *et al.*, 2010). Moreover, ethylene-vinyl acetate (EVA) and polylactide (PLA)

are important biodegradable shape polymers presently in use (Huang *et al.*, 2012; Lendlein, A. and Langer, 2002; Mather *et al.*, 2009; Xue *et al.*, 2010).

Recent works have studied the way to overcome the disadvantages of these materials, such as their low thermal conductivity and poor mechanical properties, at the same time developing biodegradable materials with low toxicity in order to open up the potential for resorbable medical implants such as sutures and stents (Huang *et al.*, 2012). Pierce *et al.* (2008) developed a new class of thermoplastic polyester urethanes containing novel soft segments that presented low cytotoxicity and showed an extraordinarily high elongation at break of greater than 2100%. In other work, Knight *et al.* (2008, 2009) prepared a new biodegradable polyurethane system that incorporates a PLA soft block with a hard block bearing the inorganic polyhedral oligosilsesquioxane (POSS) moiety. The design of this hybrid organic–inorganic moiety allowed precise control of properties such as biodegradability in the polymer network chain. In particular, the increase of POSS content decreased the degradation rate and enhanced the crystallinity as a result of its hydrophobic and nonhydrolysable properties.

At the same time, shape memory composites have been developed to switch temperature and mechanical properties, using different inorganic fillers (Meng. and Hu, 2009; Ratna and Karger-Kocsis, 2008). Zheng *et al.* (2006) incorporated hydroxylapatite into PLA, obtaining a composite that showed good biodegradation, biocompatibility and excellent shape memory properties. Additionally, Auad *et al.* (2008) reinforced shape memory polyurethanes using nanocellulose crystals by suspension casting, with the aim of increasing the stiffness of these materials, thus enhancing their competitiveness. In general, tensile modulus and strength are significantly increased in line with the nanocellulose content (for example, a 53% increase tensile modulus is associated with 1 wt% cellulose nanocrystals). These advances in the design of SMPs are expected to have an impact on future applications of these materials in the field of medical devices (Auad *et al.*, 2008).

12.3.3 Stimuli-responsive nanoparticles

Currently, the important advance of nanotechnology has allowed the development of stimuli-responsive nanomaterials with great potential, particularly in the field of medical devices. In this respect, intelligent nanomaterials offer an exciting new approach, and potential applications, such as the development of biosensors, microfluidic devices, point-of-care assays, diagnosis of diseases by imaging techniques and their treatment by hyperthermia. The configuration of nanomaterials can vary hugely, from micelles, NPs and dendrimers to hybrid colloidal core–shell particles with active surfaces adapted, for example, to the detection of specific biomolecules. Moreover,

these nanomaterials can respond to different external stimuli such as temperature, pH, electric field, biological agents and magnetism, giving them great potential (Cabane *et al.*, 2012; Huang and Juang, 2011; Stuart *et al.*, 2010; Yang and Liu, 2011).

The fabrication of nanomaterials and, particularly, intelligent NPs, can be performed using different methods based on three different strategies (see Table 12.1). One strategy is based on the use of previously synthesized polymers employing different approaches such as coacervation and precipitation methods (Chuang *et al.*, 2009; Filippov *et al.*, 2008; Koňák *et al.*, 2007; Sarmento *et al.*, 2007), layer by layer (LbL) polymeric shell formation (Radt *et al.*, 2004; Such *et al.*, 2007; Wong *et al.*, 2008) or grafting reactions of polymers onto inorganic NPs (Lai *et al.*, 2007, 2009; Tu *et al.*, 2007; Zhang *et al.*, 2007a). The second strategy is heterogeneous polymerization, which has become a widely used method in recent years. It can be performed by precipitation polymerization (Berndt *et al.*, 2006; Jones and Lyon, 2000), emulsion polymerization (Arias *et al.*, 2008; Gan and Lyon, 2003) and dispersion polymerization (Rieger *et al.*, 2009). The third attractive strategy is physical adsorption, which is based on the synthesis of block copolymers that can form polymeric micelles by self-assembling mechanisms (Chen *et al.*, 2009; Jiang and Zhao, 2008; Theato, 2008; Yusa *et al.*, 2009).

In general, the synthesis of NPs focus on the building of core–shell structures with an efficient design that allows for combining high sensitivity to different external stimuli with appropriates changes in the morphology of particles that could be useful for a wide range of applications (Cayre *et al.*, 2011; Motornov *et al.*, 2010).

The morphological and structural properties of the stimuli-responsive NPs can be quite different. The most typical design of NPs is represented by a core–shell architecture based on the self-assembly process of block copolymers (Stuart *et al.*, 2010). In particular, Pluronics® triblock copolymers (poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide)) are able to respond to temperature through changes in their supramolecular interactions. The combination of this system with a specific dye allows its use as a biosensor for imaging techniques (Cabane *et al.*, 2012). Moreover, block copolymers can form a bilayer membrane of polymeric vesicles that presents a spherical shell structure (Cabane *et al.*, 2012; Rodríguez-Hernández *et al.*, 2005). With good stability, high toughness and the possibility of tuning membrane properties, these vesicles are excellent materials for medical devices (Du and O'Reilly, 2009).

Responsive nanogels have also been of great interest in recent studies because of their versatile properties (Ballauff and Lu, 2007). Most nanogels are composed of PNIPAM and its different copolymers, with a core–shell structure and with potential application in microfluidic devices, biosensing and molecular diagnosis (Kesselman *et al.*, 2012; Miyata *et al.*,

Table 12.1 Methods of preparation of polymeric nanoparticles

Technology	Method		Examples	References
Pre-synthesized polymers	Coacervation and precipitation	Simple	PNIPAM nanoparticles	Koňák *et al.*, 2007
			poly(N-methacryloyl-l-valine) nanoparticles	Filippov *et al.*, 2008
		Complex	Chitosan nanoparticles	Chuang *et al.*, 2009
			Polysaccharides nanoparticles	Sarmento *et al.*, 2007
	LbL		PNIPAM microgels particles	Wong *et al.*, 2008
	Polymeric shell		Poly(acrylic acid) nanocapsules	Such. *et al.*, 2007
			Gold nanoparticles into PNIPAM capsules	Radt *et al.*, 2004
	Grafting onto		PNIPAM grafted to magnetic nanoparticles	Lai *et al.*, 2007; Lai *et al.*, 2009
			PNIPAM layers grafted to silica nanoparticles	Tu *et al.*, 2007; Zhang *et al.*, 2007a
Heterogeneous polymerization	Precipitation		PNIPAM microgel nanoparticles	Berndt *et al.*, 2006
			Poly(PNIPAM-co-AA) microgel nanoparticles	Jones and Lyon, 2000
	Emulsion		NPs based on poly(butyl methacrylate) core and PNIPAM shell	Gan and Lyon, 2003
			Colloidal nanospheres of poly(butylcyanoacrylate) with a magnetite core	Arias *et al.*, 2008
	Dispersion		Pegylated NPs composed of N,N-diethylacrylamide and N,N-methylene bisacrylamide	Rieger *et al.*, 2009
Physical adsorption	Block copolymers		PNIPAM-*b*-poly(L-glutamic acid)	Theato, 2008
			Thermo-responsive glycomicelles	Chen *et al.*, 2009
			pH-responsive nanogels from block copolymers with controlled structures	Yusa *et al.*, 2009
			Temperature and pH-responsive copolymers based on poly(ethylene oxide) and poly(methacrylic acid)	Jiang and Zhao, 2008

2006; Seiffert and Weitz, 2010). Moreover, these nanogels are usually composed of an inorganic material that forms the NP core and provides precise properties for a specific application, thus improving their effectiveness. Currently, the most advanced application of nanogels is as magnetic NPs for *in vitro* and *in vivo* applications in the field of medical devices, as described below.

Polymer-based magnetic nanoparticles (mNPs)

Generally, these are composed of an iron oxide core and a polymer shell that can act to increase compatibility, interacting with the environment. This hybrid polymeric system offers unique properties and several advantages, such as controlled size, response regulation by external magnetic field and improvement of contrast in magnetic resonance imaging (MRI), with appropriate stability, biocompatibility, biodegradability and high magnetization (Huang and Juang, 2011; Medeiros *et al.*, 2010).

There are a number of methods for preparing mNPs, including polymerization in disperse media and the obtention of magnetic latex particles from preformed polymers. The high potential of these mNPs has promoted the investigation of new structural designs, such as their conjugation with specific biomolecules (Ravichandran *et al.*, 2012). For all applications, the appropriate and effective implementation of mNPs requires exhaustive control of their size. This depends on several factors, such as ionic strength of the media, its pH and type and concentration of the stabilizers. Usually, NPs with an average size smaller than 100 nm and narrow polydispersity provide a good balance of circulation time, ability to be manipulated by an external magnetic field and heat generation for hyperthermia. The superparamagnetic behaviour is also important, as the mNPs must not retain any magnetism once the magnetic field is removed (Medeiros *et al.*, 2010).

In vitro applications of mNPs include medical diagnostic systems, immunoassay, real-time detection and monitoring of biomarkers and biosensors (Lai *et al.*, 2007). In the case of immunoassay, mNPs allow rapid and easy separation. Other advantages are low interference and background noise, no need for pretreatment and the possibility of integrating these structures into miniature medical devices using nanolithography. Hoffman and coworkers developed a diagnostic system for the detection of protein biomarkers from human plasma. For this, gold mNPs were prepared and their surface was modified with a diblock copolymer based on PNIPAM, synthesized using reversible addition-fragmentation chain-transfer (RAFT) polymerization. These particles were mixed with iron oxide mNPs in human plasma. Due to the thermoresponse of the copolymer, mixed mNPs could be separated with a magnet. This system was able to efficiently purify and strongly enrich

the model biomarker protein in human plasma (Nash *et al.*, 2010). Lai *et al.* (2007, 2009) synthesized NPs that presented an Fe_2O_3 core with a layer of carboxylate-terminated PNIPAM chains as a corona on the surface, which was functionalized with biotin and subsequently with streptavidin. These particles show a dual magnetic and temperature behaviour and can be used as soluble reagents to facilitate diagnostic target isolation and assay in POC microfluidic diagnostic devices.

In the case of *in vivo* applications, NPs have to be biocompatible, non-toxic and without any tendency to aggregate. Contrast agents for MRI and the treatment of cancer through hyperthermia using mNPs have been suggested as uses (Medeiros *et al.*, 2010). Purushotham and Ramanujan prepared mNPs with a core–shell morphology by dispersion polymerization of PNIPAM in the presence of a magnetite ferrofluid (Fe_3O_4), which forms the core of the mNPs. The evaluation of the properties of these particles showed an excellent hyperthermia performance at relatively low concentrations (Huang and Juang, 2011; Purushotham and Ramanujan, 2010).

12.3.4 Intelligent surfaces

The development of different surfaces with stimuli-responsive properties has attracted research interest in recent years. Important advances in surface science and polymer technology have allowed the fabrication of a wide range of smart surfaces in which surface properties are controlled by external stimuli or by bioactive substances such as enzymes or proteins.

The number of applications for these intelligent systems has increased, particularly for medical devices, due to the improvement in nanotechnology processes and the possibility of modulating biomolecule activity, protein immobilization and other dynamic properties of biological surfaces. Some of the most interesting and promising applications of smart surfaces include highly sensitive biosensors, antifouling surfaces, micro- and nanofluidic devices, actuators and nanovalves for medical devices.

Different smart surfaces have been prepared with varying architectures and mechanisms for inducing changes in surface properties. The most important intelligent surfaces are based on stimuli-responsive polymers forming self-assembled monolayers (SAMs) and thin films, which include the use of polymer brushes and membranes. In each case, the intensity of external stimulus necessary to trigger the response of the material, the rate of this response and the amplitude and reversibility of the changes, are different, which means that specific surface architecture can be designed for each application. Smart surfaces, therefore, are increasingly relevant (Cabane *et al.*, 2012; Mendes, 2008; Stuart *et al.*, 2010).

Self-assembled monolayers (SAMs)

SAMs are ordered molecular assemblies which form spontaneously by the adsorption of an active surfactant onto a solid surface. This process is an important example of the general phenomenon of self-assembly that is characterized by the spontaneous formation of hierarchical structures from designed building blocks (Olivier *et al.*, 2011; Schreiber, 2000; Smith *et al.*, 2004; Ulman, 1996). The development of SAMs began in 1946, when Zisman described the preparation of a monomolecular layer by adsorption of a surfactant onto a clean metal surface (Bigelow *et al.*, 1946). However, the potential of self-assembly was not accepted until Nuzzo and Allara (1983) prepared SAMs of alkanethiolates on gold. These materials have permitted the development of highly flexible surfaces, with a significant increase in the molecular complexity and stability of two-dimensional assemblies (Ulman, 1996).

SAMs are formed by the association of three specific entities: head-group, end-group and backbone. These macromolecules can incorporate a wide range of groups both in the alkyl chain and at the end-group. For technological applications, most systems are based on thiol head-group derivatives or gold and silane head-group derivatives on silicon dioxide, widely used to modify the surface properties of metallic and inorganic substrates (Frank, 2004; Lahann *et al.*, 2003; Onclin *et al.*, 2005). Moreover, it is possible to incorporate active groups that specifically bind targeted biomolecules onto specific surfaces, such as DNA, antibodies, enzymes, proteins or growth factors. Therefore, a variety of surfaces with specific interactions can be produced, with a wide range of applications for medical devices due to their optimal structural, biomimetic and biocompatible properties; they are especially interesting in the development of biosensors (Olivier *et al.*, 2011).

An example of novel applications of SAMs is the development of new biological sensors, in particular, the electrochemical DNA (e-DNA) sensors that combine rapid detection, minimal power requirements and low production cost with an excellent sensitivity. These systems are based on the use of redox labelled (particularly methylene blue or ferrocene) oligonucleotide probes site-specifically attached to a gold electrode surface that becomes fluorescent upon hybridization with the target DNA sequences (Kang *et al.*, 2009; Mendes, 2008; Ricci *et al.*, 2007). Immoos *et al.* (2004) developed a signal-on sensor for DNA using a flexible PEG spacer that linked the capture and probe DNA chains to a gold electrode surface. The close proximity of the immobilized capture and probe DNA chains facilitates target binding and provides a specific detection method.

In recent years, the surfaces of polymer systems have been modified using SAMs, which is especially attractive for the fabrication of medical micro- and nanodevices due to excellent properties, including optimal flexibility.

This is one of the most promising routes for the development of better diagnostic–sensing systems. In particular, SAMs are frequently used as ultrathin organic materials in nanolithography technologies in order to improve the micro- and nanofabrication of medical devices with an optimal balance between time, cost and ease of design.

Thin films and polymer brushes

The major disadvantage of smart surfaces is that the response time of these systems is too slow for many applications. This can be solved by coating the materials with thin polymeric films which maintain the mechanical properties of the original material. Polymer thin films can be prepared using several deposition techniques of differing complexity, which allows modulation of the rate of response, from seconds to hours, and permits the design of materials for a wide range of applications. Spin coating, chemical vapour deposition, plasma deposition or electropolymerization have all been employed in the fabrication of thin polymer films (Mendes, 2008; Stuart *et al.*, 2010).

Spin coating is one of the simplest techniques for applying thin films, but it is no use for low solubility polymers (Hall *et al.*, 1998). Chemical vapour deposition solves this problem because monomers are delivered to the surface in the vapour phase, eliminating the need to dissolve macromolecules. They then undergo simultaneous polymerization, resulting in the formation of a thin film. Moreover, the substrate compatibility obtained using this method is excellent for biomedical devices such as implants, membranes and microfluidic devices (Asatekin *et al.*, 2010).

Electropolymerization is another widely used method for film deposition applicable to many materials of the conducting polymer family (Jegadesan *et al.*, 2005). Plasma polymerization involves the deposition of materials from organic precursors at temperatures below 100°C for biomedical applications. This deposition method offers some important advantages, including a high deposition rate, low consumption of chemicals and no need for solvents, as well as the ability to create a wide variety of chemical structures on different substrates. However, the use of this method for the production of biomedical devices is still in its infancy (Förch *et al.*, 2007; Friedrich, 2011).

Recently, a new type of thin film has been developed based on the use of long polymer chains that are attached chemically to a surface at sufficiently high grafting densities to create new surface functionalities that respond to environmental conditions. These films are an effective alternative for tuning the relevant surface properties of many medical devices.

The preparation of these systems based on *polymer brushes* is performed using grafting techniques. These have advantages over other approaches,

particularly the specific and controllable localization of the polymer chains on the surface creating grafted layers with high density and stability. Polymer brushes, therefore, can significantly improve control of the coverage, thickness and composition of surfaces, which is *not* possible using SAMs (Stuart *et al.*, 2010; Uhlmann *et al.*, 2006; Zhang and Han, 2010).

In the 'grafting-to' technique, end-functionalized polymer reacts from solution onto a suitable substrate surface to form polymer brushes. This method is particularly suitable for homogeneous brushes using linear polymers with a narrow molecular weight distribution. However, the amount of polymer that can be attached to the substrates is low, giving very thin layers of limited density.

On the other hand, the 'grafting-from' method uses a polymerization initiated from the substrate surface, which has initiating groups attached through covalent bonds. In this case, polymer brushes are characterized by high grafting densities with a specific degree of control over the thickness, composition and chain architecture on the surface. One possible disadvantage of this method is that the polymer brushes can be quite inhomogeneous due to the high polydispersity of radical polymerizations. However, the use of existing polymerization techniques, such as RAFT, atom-transfer free-radical polymerization (ATRP) or nitroxide-mediated radical polymerization (NMRP), is an excellent alternative for controlling the molecular properties of these polymer systems (Edmondson *et al.*, 2004; Olivier *et al.*, 2011).

The simplest case is the preparation of homopolymer brushes. For example, PNIPAM brushes have been developed for cantilever actuation and the detection of changes in solvent type, temperature and pH, promising great potential for sensing applications in microfluidic devices (Abu-Lail *et al.*, 2006; Qing and Sun, 2011). Tokareva *et al.* (2004) prepared a novel sensor for pH detection based on poly(2-vinylpyridine) polymer brushes which detected a change in pH from 5 to 2.

Another interesting possibility, which has only recently been experimentally investigated, is the development of block copolymers or mixtures of polymers to create surfaces with gradient properties such as grafting density, molecular mass and chemical composition. In the case of combining different polymeric blocks, the result is a broadening of the switching range of properties, particularly when the solvent affinities of each block are different. On the other hand, the use of incompatible polymers, for example polystyrene and poly(2-vinylpyridine), that are grafted consecutively onto the surface, allows the design of mixed brushes for changing surface composition and wetting behaviour after treatment in different solvents (Draper *et al.*, 2004; Minko *et al.*, 2005; Uhlmann *et al.*, 2006; Xu *et al.*, 2006; Zhang and Han, 2010).

12.3.5 Nanopatterning surfaces

The improvement of micro- and nanotechnologies has allowed the development of advanced miniature biomedical devices that are able to control the spatial distribution and behaviour of biomolecules at surfaces. Such devices are also capable of detecting and responding quickly to disease states at the site, thus improving quality of life for patients. In particular, the production of chemical, biological and topographical micro- and nanopatterns on surfaces has facilitated the fabrication of advanced microarrays, microfluidic devices, new biosensors and more effective diagnostic systems, using a wide range of high-resolution patterning techniques coupled with functional surface chemistry (Caldorera-Moore and Peppas, 2009; Hook *et al.*, 2009). These systems have important advantages, compared to other devices fabricated with traditional manufacturing techniques, such as better resolution and sensitivity, smaller dimensions and enhanced reliability (Ziaie *et al.*, 2004). Specific strategies for the surface patterning of biomolecules are divided into different categories: direct-writing technologies, photolithography, electron beam lithography, soft lithography and microfluidic devices. These are described below.

Direct-writing technologies are based on the movement of a print head in a defined pattern that permits injection at a precise location on a surface. This fabrication method is particularly interesting for the development of microarrays, and includes different alternatives such as contact printing and dip-pen nanolithography. Contact printing uses a robotic spotter that first dips a microscale-diameter pen into the required solution and then spots the sample onto the substrate surface at a specific location. An improvement of this technology is fabrication by non-contact printing, which is performed by ejecting nanolitre volumes of the required solution from a microcapillary onto the substrate surface at the relevant location. In both cases, resolution is limited to around 100 nm. Dip-pen nanolithography is a scanning probe nanopatterning technique in which an atomic-scale tip is used to deliver chemical or biological reagents, termed 'inks', directly to nanoscopic regions of a target substrate. This approach was developed by Mirkin *et al.* in 1999 and was first demonstrated for generating patterns based on SAMs. Here a resolution as low as 50 nm can be achieved, depending on factors such as tip geometry and the chemical nature of the ink and substrate. (Bai and Liu, 2012; Hook *et al.*, 2009; Mendes *et al.*, 2007; Olivier *et al.*, 2011).

An alternative approach to the direct-writing technique is the use of **electron beam lithography** to create biologically active and intelligent nanostructures by first patterning a pre-formed homogeneous film, and then attaching the biomolecules of interest using a beam of high-energy electrons. This technology was developed soon after the invention of the

scanning electron microscope in 1955. Electron beam nanolithography is useful for producing high-resolution features down to 5 nm. However, the processing time is slow and the cost is high (Hook *et al.*, 2009; Mendes *et al.*, 2007; Olivier *et al.*, 2011).

Photolithography is the most successful technology due to its ability to generate structured patterns with large surface areas through the irradiation of a surface by a high-energy beam. The procedure consists of coating a surface with a photosubstrate that is subsequently ablated by exposure to UV light through a mask. Typically, SAMs with a functional group capable of linking different biomolecules can be formed on the re-exposed regions. Finally, the remaining coating can be removed and antifouling materials can be deposited on the uncoated regions (Hook *et al.*, 2009). Photolithography is expensive, however, with rigorous experimental protocols and material limitations when used on non-planar surfaces, and so other technologies have been explored to complement it, including soft lithography (Hook *et al.*, 2009; Zhang and Han, 2010).

Soft lithography encompasses different methodologies that use an elastomeric stamp to transfer patterns to substrates. Different elastomers have been developed to optimize the properties of the stamp, which needs to have a high modulus and low surface energy. Polydimethylsiloxane (PDMS), polyurethane and polyimide are typical materials. Microcontact printing is a widely implemented application of soft lithography which uses a stamp to transfer an image onto a surface. Different biomolecules can be directly transferred in a controlled way onto a variety of substrates, making microcontact printing a versatile technique for many applications in the field of medical devices (Mendes *et al.*, 2007; Rogers and Nuzzo, 2005; Zhang and Han, 2010).

Finally, **microfluidics** is an important alternative approach to surface patterning, with reduced costs and simple manufacturing procedures. In this case, the surface design depends upon the manipulation and spatial control of the biomolecule through limitation of the accessible surface of the substrate by the creation of microchannels. Medical diagnostics and biosensors are two important application areas for microfluidic technology. Moreover, microfluidics has permitted the development of POC systems and applications (Hook *et al.*, 2009; Rivet *et al.*, 2010; Zhang and Han, 2010).

12.4 Applications: medical devices based on shape memory polymers (SMPs)

Smart polymers have been used in the design and construction of medical devices, with an emphasis on biosensors, bioactuators and microfluidics-based systems for enhanced diagnostics and therapy, other medical devices for cancer diagnosis and therapy, and for MIS. This section and those following review these applications. Applications of smart polymers for medical

devices include their exploitation in a number of medical fields, including cardiology, angiology, otology, nephrology, endocrinology, neurology and orthodontics. The capability of SMPs to recover their original shape after deformation, under appropriate stimulation, is especially useful for items from simple medical accessories to complex implantable devices.

SMPs, with recovery strains higher than 300% and recovery stresses between 1 and 10 MPa, are advantageous compared to other shape memory materials such as alloys, which exhibit recovery strains lower than 8% and recovery stresses approaching 1000 MPa (Small *et al.*, 2010). Moreover, SMPs can be shaped into elaborate geometries at a lower cost (Small *et al.*, 2010).

Photo-, chemo- or thermo-responsive SMPs can all be used for medical devices (Huang *et al.*, 2010a), the latter being the most popular, as their primary form may be restored simply by body heat, if designed to have the proper switching transition temperature (Lendlein and Langer, 2002). Also, polyurethane SMPs, which are moisture-responsive and are able to restore their primary form upon immersion in water, have also been proposed for certain applications (Huang *et al.*, 2010a).

Thermally sensitive SMPs represent an innovative alternative to the conventional rings implanted for cardiac valve repair. These rings are inserted in the mitral valve cavity when it fails to close correctly, reducing its diameter and so avoiding blood-flow reversal (Enriquez-Sarano *et al.*, 1995). Using SMPs, the diameter of the ring can be reduced progressively by heating following surgery, so that the device does not have an abrupt impact on cardiac function. The ring can be implanted in a secondary shape, which resembles the physiological shape of the patient's actual valve, and heat can be then applied electrically (through resistance) or magnetically (using mNPs) so that it recovers its primary shape (Small *et al.*, 2010).

SMPs have also been investigated for the reconnection of small blood vessels (microanastomosis) as an alternative to the traditional approach of stitching the two severed ends together, which requires long surgical procedures, Smela (2003) proposed polypyrrole bilayered tubes as blood vessel connectors which could be inserted in only a couple of minutes. These would be prepared under a reducing potential which, when removed, would allow the polypyrrole to return to its oxidized state and expand *in situ*, exerting a spring-like force on the vessel walls that would hold the two ends together.

A similar approach for myringotomy tubes (tubes which are aimed to keep the eardrum open) has also been proposed, with a design including petal-like bulges which unfold on each side of the eardrum to hold the self-expandable tubes in position (Smela, 2003).

SMPs have also been proposed for neuroprosthetic devices aimed at stimulating and recording nervous system function. Although this has proved to be useful using conventional materials for the alleviation of symptoms such

as those caused by Parkinson's disease or deafness (Szarowski *et al.*, 2003), the implantation of the probes causes tissue damage, producing astrocytic scars which insulate the electrodes, leading to failure. In order to overcome this limitation, Sharp *et al.* (2006) investigated the use of SMP-based neuronal probes to achieve self-deployment of the electrodes at appropriately slow rates so that post-implantation tissue damage is reduced. *In vivo* experiments in mice demonstrate that the proposed approach for slow insertion succeeds in reducing astrocytic scarring.

Another pathology that could benefit from the use of SMPs is obesity. Biodegradable self-inflating intragastric implants based on smart polymers would constitute a promising alternative to conventional intragastric balloons, which need to be inflated after delivery and removed after several months. Lendlein and Langer (2004) and Marco (2006) have proposed the use of biodegradable thermo- and pH-responsive shape memory polymers for intragastric implants, which would self-deploy upon heating to body temperature or on contact with gastric fluids to a permanent form that does not interfere with the flow of food through the gastrointestinal tract (Lendlein and Langer, 2004; Marco, 2006).

SMPs also represent a promising alternative, both technically and aesthetically, to traditional orthodontic materials for corrective braces used to move teeth into alignment. Their shape-recovery force is high, they are easily processed and their appearance can be satisfactorily tuned (Jung and Cho, 2010). Nakasima *et al.* (1991) demonstrated that stretched polynorbornene wires are capable of exerting a continuous force which is capable of moving the teeth. Moreover, they exhibited less force degradation at physiological temperatures than conventional materials. Polyurethane wires were also proposed by Jung and Cho (2010)for this purpose, showing sufficiently high long-term shape-recovery forces to correct misaligned teeth.

In the field of accessories for medical devices, Ortega and Small (2007) studied the effect of a thermally deployable SMP-based adapter for kidney dialysis needles, designed to reduce haemodynamic stress on the arteriovenous grafts. This tube-shaped adapter can be delivered through the needle and thermally deployed upon blood contact. Both computational fluid dynamics simulations and *in vitro* flow visualization measurements show that the adapter is able to redirect the needle flow so that its impact on the vascular grafts is reduced.

12.5 Applications: SMPs in minimally invasive surgery

The implantation of medical devices frequently involves complicated surgical procedures. MIS causes minimal damage to body tissues, greatly reduces the risks associated with surgical procedures, reduces trauma to the patient and accelerates their recovery. Medical devices for MIS need

to be miniaturized to permit insertion into the body, for example, through small incisions, with laparoscopes or via an endovascular route (Sokolowski, 2010). The unique properties of SMPs allow the insertion of medical devices in a compact or compressed form and their subsequent deployment to functional shape once inside the body.

SMPs represent a major opportunity for the design of MIS devices for the removal of clots from blood vessels to treat ischaemic strokes. A promising approach presented by Maitland *et al.* (2002) consists of a polyurethane-based device which can be introduced into the thrombus in a rod-like form via a catheter. When activated photothermally the device attains a coil- or umbrella-like form capable of trapping the clot, which can then be mechanically removed from the artery, together with the device (Maitland *et al.*, 2002; Metzger *et al.*, 2002).

Aneurysms, which are abnormal localized dilation or bulging of a portion of a blood vessel, are another significant cause of strokes. One of the current treatments consists of delivering a number of platinum coils into the aneurysm so that the blood in it clots, reducing the risk of rupture (Small *et al.*, 2010). However, the time needed to release all the coils during the operation can lead to complications (Maitland *et al.*, 2002), and the treatment might need to be repeated if the coils compact (Small *et al.*, 2010). A photothermally activated shape memory polyurethane device has been employed to create a faster coil release system, which has been shown to free the coil in as little as one second when heated by a laser incorporated in the device (Maitland *et al.*, 1998, 2002). As the laser heats the SMP tweezers above their transition temperature, they expand, releasing the coil, before contracting again when the temperature falls (Maitland *et al.*, 2002). In addition, foams of polyurethane-based SMPs have been studied as substitutes for current aneurysm-occluding materials. Again, the foams can be compressed for insertion by minimally invasive procedures and restored to their required size once inside the body (Sokolowski, 2010; Sokolowski *et al.*, 2007). *In vivo* experiments have shown that the open cellular structure of SMP foams favour neointimal formation (Small *et al.*, 2010).

Another attractive application area for SMPs is in medical stents, either for treating arterial stenosis (Small *et al.*, 2010) and preventing strokes (Behl and Lendlein, 2007), or for urologic procedures (Farokhzad *et al.*, 2006). Stents are little tubular constructions that are placed inside blood vessels or other body conduits to prevent their obstruction or narrowing, thus preserving the natural flow of fluids (Nawrat, 2008; Small *et al.*, 2010). Currently, most commercially available vascular stents are made of metallic materials (Small *et al.*, 2010; Sokolowski, 2010). However, SMPs are able to store and recover larger strains (Huang *et al.*, 2010a; Yakacki *et al.*, 2008); so they could be reduced to a smaller size for delivery to the lesion using a smaller catheter, followed by controlled deployment at body temperature (Yakacki

et al., 2007). Their reduced stiffness would also facilitate delivery throughout the complex vessel network (Small *et al.*, 2010). Moreover, these polymers offer the possibility of incorporating drugs to reduce rejection responses and common restenosis or thrombosis after implantation, without the need for any additional coating steps during fabrication (Mather *et al.*, 2009). They also have an advantage in terms of production costs, which could be more than 50% lower than conventional metallic stents (Sokolowski, 2010).

Block copolymers based on hard segments of crystallizable poly((R)-3-hydroxybutyrate-co-(R)-3-hydroxyvalerate) (PHBV) and switching segments of hyperbranched three-arm poly(3-caprolactone) were proposed by Xue *et al.* (2010) as biodegradable SMPs for fast, self-deploying stents. Those containing copolymer with 25 wt% PHBV demonstrated almost complete self-expansion at 37°C within just 25 s, which is much faster than current self-deployable stents.

Gall *et al.* (2005) also demonstrated the shape recovery of stent prototypes made of thermo-responsive acrylate-based SMPs at physiological temperature. And Wache *et al.* (2003) proposed a drug-eluting stent based on thermoplastic shape memory polyurethane showing steady drug release characteristics.

For the optimization of SMP-based stents, their deployment process after insertion can be simulated. Previous simulations of braided stents based on shape memory polyurethanes showed gradual expansion with increasing temperature up to physiological temperature, so that abrupt overpressures to the arteries are avoided during the process (Hyun Kim *et al.*, 2010).

In the field of urology, biodegradable SMP materials may be used to develop drug-eluting stents which can be introduced into the urinary tract using an endoscope to act as depots of drugs such as antibiotics or alkalizing agents. The stents would subsequently degrade, removing the necessity for any removal procedures (Farokhzad *et al.*, 2006).

It may be possible to use SMPs to accomplish intricate mechanical deformations automatically. For instance, SMPs could help in suturing small incisions, such as those used in MIS, with just enough force to close the wound but insufficient to cause damage that might lead to necrosis in the surrounding tissue (Leng *et al.*, 2009). Thus, an elongated, thermally activated SMP fibre could be applied loosely by the surgeon before automatically tightening as it warms, tying the knots itself (Farokhzad *et al.*, 2006). If parameters such as the composition of the polymer, the elongation of the fibres and the loose suture performance are controlled, optimum stress may be achieved (Lendlein and Langer, 2002; Leng *et al.*, 2009). Furthermore, if the polymeric material used is biodegradable, no stitch removal would be needed.

Lendlein and Langer (2002) demonstrated this concept using multiblock copolymers based on hard segments of crystallizable oligo(p-dioxanone) diol and switching segments of oligo(ε-caprolactone)diol. Fibres of this

material were heated to elongate them by 200% and subsequently cooled to fix their secondary shape. They were then used to loosely suture an abdominal incision in a rat, achieving wound closure when they reached body temperature.

12.6 Applications: medical devices for cancer diagnosis and therapy

Cancer is one of the most worrying classes of diseases affecting the society nowadays due to its high morbidity and mortality rates. Smart polymers can contribute to the development of new techniques that allow its early diagnosis, and provide alternatives to the currently available therapies, improving their effectiveness and reducing their severe side effects.

12.6.1 Thermal ablation of tumours

Hyperthermia (also known as thermoablation) has been proposed as a non-toxic alternative to chemotherapy or radiotherapy for the treatment of malignant tumours (Ito *et al.*, 2004; Medeiros *et al.*, 2010), allowing the treatment of such tumours located in vital areas of the body where surgery is not possible (Huang and Juang, 2011). mNPs have attracted extensive attention for hyperthermia treatments, as they have the potential to be specifically delivered to the tumour cells and remotely heated once there by an external magnetic field (Shinkai, 2002). However, preventing unwanted heating of the surrounding healthy tissue due to incorrect localization of the particles is still one of the most important limitations of this technique (Tasci *et al.*, 2009). A promising approach for targeting the mNPs specifically at tumours (targeted hyperthermia) is to coat them with smart polymers. For instance, antibody-conjugated polymers with enough specificity for exposed tumoural antigen could facilitate targeted delivery, allowing cellular uptake of the mNPs by the cancer cells (intracellular targeted hyperthermia) (Huang and Juang, 2011).

Furthermore, if the polymeric coating is pH- or temperature-responsive, controlled drug release from drug-loaded mNPs could also be attained by means of conformational changes of the polymer chains in response to appropriate stimuli. This approach would combine drug targeting, controlled drug release and hyperthermia (Purushotham and Ramanujan, 2010), with potential synergistic therapeutic effects (Bull, 1984; Wust *et al.*, 2002).

Purushotham and Ramanujan (2010) presented a case study in which iron oxide (Fe_3O_4) mNPs were coated with a thermo-sensitive polymer, PNIPAM, and used for simultaneous magnetic hyperthermia and controlled

chemotherapeutic release; by increasing the temperature above the LCST of the polymer, faster drug release was achieved. Other smart polymeric coatings such as poly(D,L-lactide-co-glycolide) (Koppolu *et al.*, 2010) or poly((2-dimethylamino)ethylmethacrylate) (Zhou *et al.*, 2009) have also been exploited to obtain different drug release profiles.

12.6.2 Magnetic resonance imaging diagnosis

Smart polymeric coatings for mNPs have also been a source of interest in the field of diagnostics, especially for MRI, which is a very useful non-invasive technique for obtaining high-resolution images for the detection of tumours (Jun *et al.*, 2005). To improve the image contrast between healthy and cancerous tissue in MRI, mNPs are commonly used as contrast agents (Na *et al.*, 2009). In fact, iron oxide NPs have been shown to be effective in diagnosing cancer *in vivo* (Lee *et al.*, 2006). However, bioactive coatings which are able to recognize specific ligands help in attaining enhanced targeted imaging, as the NP accumulation in particular tissues is more effective (Huang and Juang, 2011). Hu *et al.* (2006) have demonstrated that biocompatible PEG-coated mNPs conjugated with anti-carcino-embryonic antigen monoclonal antibody rch 24 can successfully target tumour cells *in vivo*, suggesting their potential application as contrast agents for MRI.

Additionally, the potential application of smart polymer-coated mNPs for both diagnosing and treating tumours can be combined to develop simultaneous diagnosis and therapy strategies within the same system (Medeiros *et al.*, 2010). This combination of targeted delivery, MRI diagnosis and magnetic hyperthermia or controlled drug release will greatly enhance treatment effectiveness and reduce damage to healthy tissue (Huang and Juang, 2011).

12.7 Applications: biosensors for diagnostic medical devices

Biosensors are devices capable of recognizing biological events and turning them into measurable signals (Mendes, 2008; Ponmozhi *et al.*, 2012; Soper *et al.*, 2006). Smart polymers are particularly useful in biosensing applications for various reasons. Their intrinsic responsiveness to different stimuli, especially to those which occur in physiological conditions, enables effective transduction (Stuart *et al.*, 2010) by means of conformational or physicochemical shifts. Many can easily incorporate biological material or biomimics as receptors. Furthermore, macromolecular structures allow easier integration into detection devices than their small-molecule counterparts (Hu, 2010).

Because a number of diseases are characterized by changes in the concentration of certain analytes, or in physical variables such as temperature or pH, biosensors can play a significant role in clinical diagnostics and forensic analysis (Cabane, 2012; Mendes, 2008), They permit the early detection of specific chemicals or biomarkers in the body and make possible the continuous monitoring of variations in selected biological parameters. Medical devices based on smart biosensors can, therefore, substitute more traditional diagnostic tools such as chromatography, mass spectroscopy or ELISA (Enzyme-Linked ImmunoSorbent Assay) immunoassays (Vaddiraju *et al.*, 2010), which usually require a lot of equipment, take a long time and are relatively expensive.

Numerous biosensors based on smart polymers with potential medical applications have been proposed. These include pH and temperature sensors (Cabane, 2012) and biosensors for the detection of different analytes and biomolecules such as carbon dioxide (Herber *et al.*, 2004), oxygen (Deligkaris *et al.*, 2010), ammonium (Kwan *et al.*, 2005), urea (Ohnishi *et al.*, 2010), lactate (Kwan *et al.*, 2004a), alanine (Kwan *et al.*, 2004b), pyruvate (Ponmozhi *et al.*, 2012), glutamate and glutamine (Ponmozhi *et al.*, 2012), 3-hydroxybutyrate (Kwan *et al.*, 2006), creatin and creatinine (Ponmozhi *et al.*, 2012) and organo-phosphorus compounds (Argentiere *et al.*, 2012), among others.

12.7.1 Biosensors based on physical variables

Herber *et al.* exploited a pH-sensitive hydrogel to design a pH sensor for the detection of carbon dioxide gas inside the stomach, in order to diagnose gastrointestinal ischaemia (Herber *et al*., 2005b). This disorder is caused by a deficient blood flow to the stomach and intestines resulting in insufficient delivery of oxygen and nutrients to these organs, with the consequence of increased gastric carbon dioxide levels (Herber *et al.*, 2005a). This smart hydrogel, based on poly(dimethylaminoethyl methacrylate) (PDMAEMA), was introduced in a bicarbonate solution and enclosed inside a porous cover with a pressure sensor. The pH reduction due to the reaction of CO_2 with the bicarbonate solution results in swelling of the hydrogel, but as the volume is restricted by the cover, pressure is generated. This pressure can be related to the partial pressure of carbon dioxide in the stomach without the need for a reference electrode (Herber *et al.*, 2003,2004).

Tagit and coworkers developed surfaces which can act as nano-thermometers using quantum dots (QDs) attached to PNIPAM polymer brushes grafted onto a gold substrate. The luminescence of these QDs could be quenched by increasing the temperature above the LCST of PNIPAM, and recovered by decreasing it (Tagit, 2009).

12.7.2 Glucose biosensors

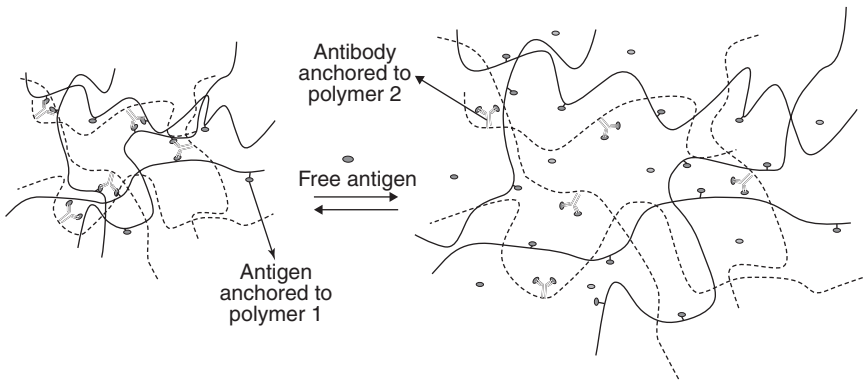
Among the many different analyte-responsive polymers that have been designed for biosensing applications, smart polymers which respond to glucose have received significant attention due to their potential use for the management of diabetes mellitus, one of the main global health problems leading to disability and death (Tierney *et al.*, 2000; Wang, 2001). Most glucose biosensors based on smart polymers make use of the enzymatic reaction of glucose with oxygen, catalysed by GOx, to yield gluconic acid and hydrogen peroxide (Ravichandran *et al.*, 2012; Wang, 2001), a reaction that was first conceived for glucose biosensors by Clark and Lyons (1962). Thus, polymers which respond to either of these products can be incorporated into a glucose-detecting device, including pH-responsive polymers, as the gluconic acid by-product lowers the pH. For example, various polyelectrolytes such as chitosan or poly(dimethyldiallyl ammonium chloride) have been used in glucose biosensors (Chaterji *et al.*, 2007).

A different approach has been proposed by Brownlee and Cerami. They reported a glucose-responsive system based directly on the competitive binding of glucose with glycopolymer–lectin complexes (Brownlee, 1979). Recently, Huang *et al.* (2010b) presented a biosensor based on changes in the dielectric properties of a polymer due to its specific, reversible binding to glucose. The proposed microsensor included a solution of poly(acrylamide-ran-3-acrylamidophenylboronic acid) placed between two electrodes. The resistance of this polymer changes when it binds to glucose, so that the capacitance measurement allows glucose detection (Huang *et al.*, 2010b).

Several glucose monitoring kits based on biosensors are already being developed commercially (Ponmozhi *et al.*, 2012). Commercially available systems are designed to be worn by the patients for several days and can take readings as frequently as every minute, allowing data storage and analysis. High and low threshold glucose level alerts can also be set (Wilson and Gifford, 2005).

12.7.3 Biosensors for the detection of other analytes

Biosensors sensitive to very specific analytes are those based on antigen-sensitive polymers, as they focus on highly specific antigen–antibody interactions. Miyata *et al.* (1999a, b) prepared antigen-responsive hydrogels using the specific binding between an antigen and the corresponding antibody as the cross-linking mechanism, with both grafted to polymer chains. Thus, in the presence of free antigen, competitive binding to the grafted antibodies breaks some of the cross-links, inducing swelling of the hydrogel. Likewise, in the absence of free antigen the hydrogel shrinks, displaying an additional shape memory behaviour). The change in volume of these



12.3 Change in volume of an antigen-sensitive hydrogel in response to the target antigen.

antigen-sensitive hydrogels, or the pressure exerted by them if confined, can be measured and exploited for bioanalysis or diagnosis of disorders which are characterized by the presence of a specific antigen (see Fig. 12.3).

A similar approach has been used more recently by Miyata *et al.* (2006) to prepare tumour marker-responsive hydrogels. These polymeric networks, prepared by biomolecular imprinting, were conjugated with lectins and antibodies so that they exhibited volume changes in response to antifreeze glycoprotein (AFP), a glycoprotein which is widely used for the serum diagnosis of primary hepatoma.

Another exciting development for specific biomolecule detection is the so-called e-DNA sensor, which is composed of DNA stem-loops labelled with electroactive moieties and grafted to a surface which acts as an electrode. Detection is based on the alteration of the Faradaic current between the redox species and the electrode. This is due to structural rearrangement upon hybridization of the DNA strain with the target sequence (i.e., specific complementary DNA sequence), which causes a significant increase in the distance between the elements. Thus, the measurable reduction of the current can be related to the presence of a specific DNA sequence (Mendes, 2008). Different DNA or RNA sequences can be selected against different targets with high specificity and affinity (Hermann and Patel, 2000) in order to diagnose various disorders.

12.8 Applications: biosensors and actuators for enhanced diagnostics and therapy

Smart polymer-based biosensors are used not only for diagnosis but also for therapy. For instance, they can be helpful in enhancing response to some medical treatments which require drug monitoring to avoid side effects that

may occur if systemic concentrations exceed certain levels. This is the case for phenytoin, cyclosporine, lithium, theophylline or gentamicin, among others (Bengtsson, 2002; Chan and Beran, 2008; Hitchings, 2012; Kahan *et al.*, 2002; Magis-Escurra *et al.*, 2012; McKee *et al.*, 1992; Shaw *et al.*, 1999;). As the standard method for therapeutic drug monitoring requires frequent blood samples, which must be then analysed, real-time biosensing can save time, thus improving treatment.

Furthermore, diagnosis and therapy can be combined in medical devices which use smart biosensors and actuators, via closed-loop systems. Open-loop systems, described above, are able to monitor systemic levels of specific biomarkers or drugs but are not capable of readjusting the treatment by themselves. Closed-loop systems, on the other hand, are able not only to detect imbalances in specific analytes, but also to automatically respond to them (Hillberg *et al.*, 2005).

Actuators are able to transform environmental stimuli into mechanical responses (Argentiere *et al.*, 2012). As the presence of certain molecules triggers a conformational or chemical change in smart polymers, these materials can be used as actuators or as combined sensors–actuators (Deligkaris *et al.*, 2010). To date, the most exploited response to obtain the autonomous functionality required for actuators has been the volume shifts of pH- and temperature-sensitive hydrogels (Argentiere *et al.*, 2012).

Apart from their use in biosensors for diagnostics and glucose monitoring, glucose-responsive polymers also have a potential application in insulin delivery. For example, a pH-responsive hydrogel containing GOx would respond to the drop in pH in the presence of increased glucose levels *in vivo* by collapsing or swelling, and this structural change could automatically facilitate the release of previously-entrapped insulin (Ravichandran *et al.*, 2012).

An example of analyte-responsive polymers used for biosensor-based actuating applications is glutathione-sensitive gels. Glutathione is a tripeptide with very important roles in cells, such as protection of erythrocytes from oxidative damage and maintenance of overall cellular redox homeostasis. Drug delivery devices sensitive to this metabolite can be useful for controlled delivery of therapeutics to specific cell compartments (Chaterji *et al.*, 2007). Bulmus *et al.* (2003) synthesized a glutathione-reactive and pH-sensitive smart terpolymer for controlled endosomal release of enzyme-susceptible therapeutics. This could be useful in gene and antisense therapies, as well as for vaccine development. The copolymer incorporated pyridyl disulphide acrylate (PDSA), a monomer that allows conjugation of the polymer with thiol-containing biomolecules, and their subsequent release in the presence of glutathione, once inside the cytoplasm. It also contained methacrylic acid and butyl acrylate as comonomers to give pH-sensitive, membrane-disruptive properties (Bulmus *et al.*, 2003; El-Sayed *et al.*, 2005).

12.9 Applications: microfluidics-based biomedical devices

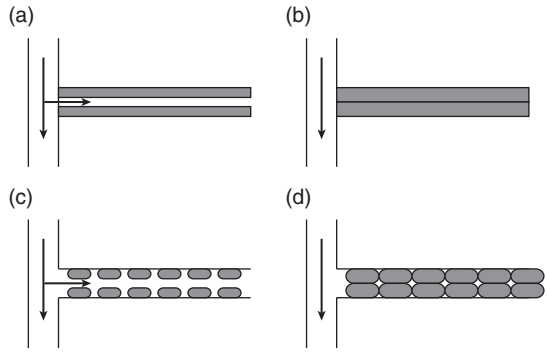
A more sophisticated approach for closed-loop medical devices able to monitor systemic levels of certain analytes and release bioactive components involves a feedback control logic unit to analyse the data acquired from the biosensors and regulate the action of the actuators. These integrated devices can be created using the so-called microfluidics technology. Microfluidics involves the miniaturization of devices and their components in order to work with minute amounts of fluid (Argentiere *et al.*, 2012). The advantages of microfluidics-based medical devices include fast and repeatable analysis, low reagent consumption, small sample requirements, low power consumption and easy automation (Sia and Kricka, 2008). Additionally, the downscaling of smart polymeric systems such as hydrogels helps increase the response rate, as this is limited by diffusion of the physico-chemical signals through the pores of the polymeric network (Chaterji *et al.*, 2007).

Practical application of these systems in the biomedical field, including for diagnostic chips and drug delivery devices (Eddington and Beebe, 2004), relies on accurate control over the transport of bioactive molecules. Although the flow of fluid through the device can be controlled by the rigid structure of the channels, it is necessary to include some components, such as microvalves and micropumps, to obtain self-regulated flow control (De Saint Vincent and Delville, 2012).

12.9.1 Microvalves

Microvalves are crucial components for microfluidic systems as they are employed to turn on, turn off and regulate the flow of liquids and at certain signals. Smart polymers are very attractive for the construction of these actuator-like elements. For example, a stimuli-responsive hydrogel properly placed inside a microfluidic channel can automatically open or close the path for fluid flow, or even regulate its cross-section when required, without the need of external control or power sources, by swelling or shrinking according to environmental conditions (Tokarev and Minko, 2009b).

Numerous smart hydrogel-based microvalve configurations have been proposed (see Fig. 12.4), including one interesting design presented by Yu and coworkers which mimics naturally occurring venous check valves, giving the capability to restrict back-flow under the correct stimulation. The pH-responsive hydrogel used in this work was obtained by simultaneous photopolymerization of a prepolymer mixture based on 2-hydroxyethyl methacrylate (HEMA), acrylic acid, ethyleneglycol dimethacrylate (EGDMA) and Irgacure® as a photoinitiator (Yu, 2001).



12.4 Two different configurations for smart hydrogel-based microvalves. Schematic smart channel design with a strip (a) of hydrogels which swell to close the channel, (b) under a given stimulus, or multiple posts of hydrogels (c) that swell to close the channel (d). (*Source*: Reprinted by permission from Elsevier: (Eddington, D.T. and Beebe, D. J. (2004) 'Flow control with hydrogels', *Advanced Drug Delivery Reviews*, 56, 199–210, copyright 2004); http://www.elsevier.es.)

Microvalves that respond to different stimuli can be fabricated from suitable hydrogels. For instance, Geiger *et al* (2010) developed a device using a thermally sensitive, hydrogel-based microvalve which closes below its LCST (32°C) but allows flow above it due to shrinking. Alternatively, Beebe *et al.* (2000) achieved flow control by spattering a pH-sensitive hydrogel along the microchannels of a microfluidic device so that fluids could flow only at appropriately low pH levels.

12.9.2 Micropumps

Micropumps are other essential components in microfluidics, as they promote fluid flow. In order for the fluids to be driven, actuators capable of generating movement are needed; so flow actuation can be pursued by exploiting volume-expansion of hydrogels. For instance, temperature-responsive poly(HEMA-co-DMAEMA) hydrogels were used by Agarwal *et al.* (2005) to build autonomous micropumps which would start pumping fluids at high temperatures but would stop doing so as the temperature decreased. The hydrogel was used to control a metallic rotor, actuation of which induced a pressure change in the microchannels, which was exploited as the pumping force.

A fascinating approach is being investigated to exploit the Belousov–Zhabotinsky reaction, which induces autonomous oscillations in the redox potential of a medium, to drive spontaneous peristaltic motion of hydrogels by controlling their volume shifts (Argentiere *et al.*, 2012). Murase *et al.* (2008)

copolymerized NIPAM, ruthenium tris(2,2′-bipyridine) (Ru(bpy)3) and 2-acrylamido-2-methylpropanesulfonic acid and achieved transportation of a cylindrical gel exploiting the propagation of the resulting peristaltic wave.

Hara and Yoshida (2008), in an attempt to extend the application of these artificial muscle-like actuators under biological conditions, synthesized a quarternary copolymer which incorporated methacrylamidopropyltrimethylammonium chloride as an oxidant supplier in addition to the aforementioned three monomers, so that self-oscillation could be achieved under physiological conditions where only malonic acid is present. There is potential for these self-oscillating gel actuators with tunable periodicity (Maeda *et al.*, 2008) to be used for peristaltic micropumps and novel biomimetic applications.

12.9.3 Integrated microfluidic systems

Both miniaturized analyte sensors and microsystems for drug delivery have been demonstrated, and the integration of both functionalities within one medical device is the next natural step. Smart polymer-based sensors, actuators, microvalves, micropumps, etc., can be combined in a single microfluidic system to construct medical devices with applications in diagnostics and/or treatment, and first attempts at developing such LOC devices are currently underway.

Huang *et al.* (2007) developed a preliminary microfluidic system capable of automatic, real-time glucose sensing and subsequent insulin injection, if necessary. It included PDMS-based micropumps, microvalves and microchannels, an insulin reservoir, a flow sensor and glucose sensors, along with a control circuit system, a compressed air source and the fingerstick needles required for blood sampling and insulin injection. The glucose biosensor was fabricated by electropolymerization of pyrrole in the presence of GOx. After oxidation of glucose by the GOx, the hydrogen peroxide by-product was further oxidized to oxygen in the presence of a platinum electrode, and an amperiometric method was used to transduce this latter reaction into a current signal (Huang *et al.*, 2007).

Microfluidics represents a useful tool for POC diagnostics too. POC systems can permit the performance of rapid, reliable and inexpensive diagnostic tests without the need to move to a clinical laboratory (Sia and Kricka, 2008), with the possibility of using different biomarkers within one device for multiplexed immunoassays (Ng *et al.*, 2010). Thus, they can help reduce the costs of screening for disease prevention, enhance patient observation to improve disease detection and improve treatment monitoring (Soper *et al.*, 2006).

As antigens manifesting a disease are often very dilute in body fluids, the first challenge in current immunoassays is to achieve clinically relevant

limits of detection for biomarkers. This involves finding a simple system to purify and concentrate them prior to analysis. Hoffman and coworkers developed an ingenious microfluidic toolkit for processing relevant biomarkers based on mNPs coated with antibody-conjugated temperature-responsive PNIPAM. Upon gentle heating, the biofunctionalized PNIPAM chains undergo a conformational change, causing the mNPs to aggregate. After aggregation they can only be separated by a small magnetic field, and the labelled antigen can then be released and assayed by lowering the temperature again (Lai *et al.*, 2007). If different antibodies can be conjugated to smart mNPs responding to different stimuli, for instance using thermo-responsive polymers with different LCSTs, separation and assay of various biomarkers can be achieved by sequentially applying these stimuli.

This thermally induced phase separation immunoassay has been extended by grafting PNIPAM to porous nylon membranes on microfluidic cards. At temperatures above the LCST of PNIPAM, the polymer–antibody conjugates are aggregated and retained on the modified filters, while other proteins can pass through. Below that temperature, the captured and labelled antigens are released as a concentrated pulse (Golden *et al.*, 2010; Nash *et al.*, 2010).

12.10 Conclusion and future trends

Smart polymers have played an important role in the development of a variety of medical devices, including biosensors, bioactuators, microfluidics-based systems, components for thermal ablation, elements for MRI and so on. All these devices are intended to improve diagnosis and therapy, achieving more convenient and efficient disease management, with benefits for both patients and health care professionals.

Progress in the development of smart polymers has significantly intensified in recent years, leading to the development of more efficient and advanced medical devices that can help detect diseases in the early stages and aid treatment, with minimal side effects. Research here has focused on the study and optimization of the most selective and specific changes in properties of stimuli-responsive polymers, and also on obtaining self-assembled systems, from NPs or vesicles to complex and hieratical surfaces in supramolecular assembly architectures (Cabane, 2012).

One particular and attractive application for smart polymers is in minimally invasive surgery. As this field aims to cause minimal damage to body tissues during surgical procedures, miniaturized medical devices capable of self-deploying after implantation through small incisions have been designed. SMPs represent a major opportunity to develop such devices which can, under specific stimuli, recover their original shape after being

compressed. There are some biomedical products already benefiting from the unique properties of SMPs, and many others are currently under development (Sokolowski, 2010). These materials have demonstrated adequate thermomechanical and shape-recovery characteristics to be successful as medical devices; regulatory issues and medical-grade manufacturing challenges are the major limitations to their practical application (Huang *et al.*, 2012; Small *et al.*, 2010).

Smart polymers are now becoming an indispensable tool for the functionalization of mNPs for both **diagnosis and treatment** of various diseases. Most applications of smart polymer-based mNPs have demonstrated good efficiency *in vitro* (Medeiros *et al.*, 2010), but further research needs to be done to achieve their application in practice. The utilization of the functionalized NPs for the thermal ablation of tumours, or for improving contrast for MRI diagnosis, are two very promising applications that need to be explored further.

Currently, clinical applications of stimuli-responsive polymers and assemblies are limited due to the high requirements for the final integration of medical devices inside the human body (Chaterji *et al.*, 2007). *In vivo* applications demand biocompatible and biodegradable polymers to prevent toxicity problems in patients. The most widely used smart polymer in biomedical devices is PNIPAM. However, other polymers are being investigated to provide attractive alternatives (Chan *et al.*, 2012). In addition to this, the progress of nanotechnology and supramolecular chemistry are important tools to enhance the interaction between medical devices and the human body, to prevent cascaded immune responses and to accurately control the size, charge, flexibility and shape of polymeric systems. The development of nanopatterning approaches is of particular interest in the development of miniature devices to increase the scope and application of smart polymers in medical devices (Bai And Liu, 2012; Caldorera-Moore and Peppas, 2009; Stuart *et al.*, 2010).

Biosensor smart polymer-based devices have undergone enormous development as they hold the promise of simple autonomous, rapid and relatively inexpensive sensing of medically relevant stimuli for real-time monitoring of physiological parameters. Biosensors are becoming more and more complex and useful, and some commercial devices have already reached the market (Ponmozhi *et al.*, 2012). However, work is needed to improve the durability of implantable biosensors, as currently they can be worn by the patient for only a few days. Additionally, such devices can be further miniaturized with the aid of microfluidics and microelectronics technologies. Although there are a number of biosensors capable of monitoring many different analytes, new strategies should be designed to extend the range of biomolecules which can be detected.

Likewise, research on smart polymers for **bioactuators** seems to be in the right direction to achieving effective and functional transformation of environmental stimuli into controlled mechanical responses that can be used for the development of smart drug delivery systems, biomimetic microfluidic components such as microvalves or micropumps, and implantable medical devices. In fact, several examples of smart polymer-based actuators are already being commercialized, such as blood vessel connectors or cochlear implants (Smela, 2003). Nevertheless, the actuation mechanisms should be further studied, as some particular behaviours are not fully understood yet. Although the demonstrated functionality is sufficient for their application, a deeper understanding of the relationship between the physico-chemical nature of the smart polymers and their actuating performance will help more directed design for a particular application.

Miniaturized platforms incorporating both biosensing and bioactuating functionalities within one integrated microfluidic device are particularly attractive because, instead of relying on simple diffusion of analytes, they allow accurate control over the transport of bioactive molecules, leading to faster response and improved sensitivity (Ng *et al.*, 2010). Thus, they represent a major opportunity in both drug delivery and POC diagnostics. Furthermore, they render it possible to use different biomarkers within one device for multiple immunoassay, although fabrication of these sophisticated devices remains a challenge which needs further development of microfluidics and patterning techniques (Argentiere *et al.*, 2012; Chaterji *et al.*, 2007). Eventually, real-time multi-analyte quantification using these devices should be feasible. Another challenge to be addressed in this field is further reduction of the complexity and size of devices to reduce cost and improve convenience.

Smart polymers include a wide range of macromolecular materials which can be conformed in a variety of configurations using many different techniques. They can also be exploited for a seemingly limitless number of medical applications. Those described above are just an illustration of what can be achieved. An exciting future in medical devices is expected for smart polymeric systems.

12.11 References

Abu-Lail, N. I., Kaholek, M., LaMattina, B., Clark, R. L. and Zauscher, S. (2006) 'Micro-cantilevers with end-grafted stimulus-responsive polymer brushes for actuation and sensing', *Sensors and Actuators B: Chemical*, **114**, 371–378.

Agarwal, A. K., Sridharamurthy, S. S., Beebe, D. J. and Jiang, H. (2005) 'Programmable autonomous micromixers and micropumps', *Journal of Microelectromechanical Systems*, **14**, 1409–1421.

Anderson, D. G., Burdick, J. A. and Langer, R. (2004) 'Smart biomaterials', *Science*, **305**, 1923–1924.

Argentiere, S., Gigli, G., Mortato, M., Gerges, I. and Blasi, L. (2012) 'Smart microfluidics: The role of stimuli-responsive polymers in microfluidic devices', *Advances in Microfludics*, **6**, 128–154.

Arias, J. L., Ruiz, M. A., Gallardo, V. N. and Delgado, A. V. (2008) 'Tegafur loading and release properties of magnetite/poly(alkylcyanoacrylate) (core/shell) nanoparticles', *Journal of Controlled Release*, **125**, 50–58.

Asatekin, A., Barr, M. C., Baxamusa, S. H., Lau, K. K. S., Tenhaeff, W., Xu, J. and Gleason, K. K. (2010) 'Designing polymer surfaces via vapor deposition', *Materials Today*, **13**, 26–33.

Auad, M. L., Contos, V. S., Nutt, S., Aranguren, M. I. and Marcovich, N. E. (2008) 'Characterization of nanocellulose-reinforced shape memory polyurethanes', *Polymer International*, **57**, 651–659.

Bai, C. and Liu, M. (2012) 'Implantation of nanomaterials and nanostructures on surface and their applications', *Nano Today*, **7**, 258–281.

Ballauff, M. and Lu, Y. (2007) 'Smart' nanoparticles: Preparation, characterization and applications', *Polymer*, **48**, 1815–1823.

Beebe, D. J., Moore, J. S. *et al.* (2000) 'Functional hydrogel structures for autonomous flow control inside microfluidic channels', *Nature*, **404**, 588–590.

Behl, M. and Lendlein, A. (2007) 'Shape-memory polymers', *Materials Today*, **10**, 20–28.

Behl, M., Razzaq, M. Y. and Lendlein, A. (2010) 'Multifunctional shape-memory polymers', *Advanced Materials*, **22**, 3388–3410.

Bengtsson, F. (2002) '260 therapeutic drug monitorings (Tdm) in relation to compliance and co-medication in psychiatric treatment', *European Psychiatry*, **17**, Supplement 1, 3.

Berndt, I., Pedersen, J. S. and Richtering, W. (2006) 'Temperature-sensitive core–shell microgel particles with dense shell', *Angewandte Chemie International Edition*, **45**, 1737–1741.

Bigelow, W. C., Pickett, D. L. and Zisman, W. A. (1946) 'Oleophobic monolayers: I. films adsorbed from solution in non-polar liquids', *Journal of Colloid Science*, **1**, 513–538.

Blanco-López, M. C., Lobo-Castañón, M. J., Miranda-Ordieres, A. J. and Tuñón-Blanco, P. (2003) 'Voltammetric sensor for vanillylmandelic acid based on molecularly imprinted polymer-modified electrodes', *Biosensors and Bioelectronics*, **18**, 353–362.

Brownlee, M. (1979) 'A Glucose-Controlled Insulin-Delivery System: Semisynthetic Insulin Bound to Lectin', Science, 206, 1190–1191.

Bulmus, V., Ding, Z., Long, C. J., Stayton, P. S. and Hoffman, A. S. (1999) 'Site-specific polymer-streptavidin bioconjugate for pH-controlled binding and triggered release of biotin', *Bioconjugate Chemistry*, **11**, 78–83.

Bulmus, V., Woodward, M., Lin, L., Murthy, N., Stayton, P. and Hoffman, A. (2003) 'A new pH-responsive and glutathione-reactive, endosomal membrane-disruptive polymeric carrier for intracellular delivery of biomolecular drugs', *Journal of Controlled Release*, **93**, 105–120.

Bull, J. M. C. (1984) 'An update on the anticancer effects of a combination of chemotherapy and hyperthermia', *Cancer Research*, **44**, 4853s–4856s.

Byrne, M. E., Park, K. and Peppas, N. A. (2002) 'Molecular imprinting within hydrogels', *Advanced Drug Delivery Reviews*, **54**, 149–161.

Cabane, E. (2012) 'Stimuli-responsive polymers and their applications in nanomedicine', *Biointerphases*, **7**, 9.

Cabane, E., Zhang, X., Langowska, K., Palivan, C. and Meier, W. (2012) 'Stimuli-responsive polymers and their applications in nanomedicine', *Biointerphases*, **7**, 1–27.

Caldorera-Moore, M. and Peppas, N. A. (2009) 'Micro- and nanotechnologies for intelligent and responsive biomaterial-based medical systems', *Advanced Drug Delivery Reviews*, **61**, 1391–1401.

Cayre, O. J., Chagneux, N. and Biggs, S. (2011) 'Stimulus responsive core-shell nanoparticles: Synthesis and applications of polymer based aqueous systems', *Soft Matter*, **7**, 2211–2234.

Chan, A., Orme, R. P., Fricker, R. A. and Roach, P. (2012) 'Remote and local control of stimuli responsive materials for therapeutic applications', *Advanced Drug Delivery Reviews*, **65**, 497–514.

Chan, K. and Beran, R. G. (2008) 'Value of therapeutic drug level monitoring and unbound (free) levels', *Seizure*, **17**, 572–575.

Chaterji, S., Kwon, I. K. and Park, K. (2007) 'Smart polymeric gels: Redefining the limits of biomedical devices', *Progress in Polymer Science*, **32**, 1083–1122.

Chen, G., Amajjahe, S. and Stenzel, M. H. (2009) 'Synthesis of thiol-linked neoglycopolymers and thermo-responsive glycomicelles as potential drug carrier', *Chemical Communications*, 1198–1200.

Chen, Z., Hua, Z., Xu, L., Huang, Y., Zhao, M. and Li, Y. (2008) 'Protein-responsive imprinted polymers with specific shrinking and rebinding', *Journal of Molecular Recognition*, **21**, 71–77.

Cheng, Z., Wang, E. and Yang, X. (2001) 'Capacitive detection of glucose using molecularly imprinted polymers', *Biosensors and Bioelectronics*, **16**, 179–185.

Chuang, C. Y., Don, T. M. and Chiu, W. Y. (2009) 'Synthesis and properties of chitosan-based thermo- and pH-responsive nanoparticles and application in drug release', *Journal of Polymer Science*, *Part A: Polymer Chemistry*, **47**, 2798–2810.

Clark Jr., L.C. and Lyons, C. (1962) 'Electrode systems for continuous monitoring in cardiovascular surgery', *Annals of the New York Academy of Sciences*, **102**, 29–45.

De Saint Vincent, M. R. and Delville, J. P. (2012) 'Microfluidic transport driven by opto-thermal effects', In R. Kelly (ed.), *Advances in Microfludics*, In Tech, France.

Deligkaris, K., Tadele, T. S., Olthuis, W. and van den Berg, A. (2010) 'Hydrogel-based devices for biomedical applications', *Sensors and Actuators B: Chemical*, **147**, 765–774.

Draper, J., Luzinov, I., Minko, S., Tokarev, I. and Stamm, M. (2004) 'Mixed polymer brushes by sequential polymer addition: Anchoring layer effect', *Langmuir*, **20**, 4064–4075.

Du, J. and O'Reilly, R. K. (2009) 'Advances and challenges in smart and functional polymer vesicles', *Soft Matter*, **5**, 3544–3561.

Eddington, D. T. and Beebe, D. J. (2004) 'Flow control with hydrogels', *Advanced Drug Delivery Reviews*, **56**, 199–210.

Edmondson, S., Osborne, V. L. and Huck, W. T. S. (2004) 'Polymer brushes via surface-initiated polymerizations', *Chemical Society Reviews*, **33**, 14–22.

El-Sayed, M. E. H., Hoffman, A. S. and Stayton, P. S. (2005) 'Rational design of composition and activity correlations for pH-responsive and glutathione-reactive polymer therapeutics', *Journal of Controlled Release*, **104**, 417–427.

Enriquez-Sarano, M., Schaff, H. V., Orszulak, T. A., Tajik, A. J., Bailey, K. R. and Frye, R. L. (1995) 'Valve repair improves the outcome of surgery for mitral regurgitation: A multivariate analysis', *Circulation*, **91**, 1022–1028.

Farmer, T. G., Edgar, T. F. and Peppas, N. A. (2008) 'In vivo simulations of the intravenous dynamics of submicrometer particles of ph-responsive cationic hydrogels in diabetic patients', *Industrial & Engineering Chemistry Research*, **47**, 10053–10063.

Farokhzad, O. C., Dimitrakov, J. D., Karp, J. M., Khademhosseini, A., Freeman, M. R. and Langer, R. (2006) 'Drug delivery systems in urology-getting 'smarter', *Urology*, **68**, 463–469.

Filipcsei, G., Fehér, J. and Zrínyi, M. (2000) 'Electric field sensitive neutral polymer gels', *Journal of Molecular Structure*, **554**, 109–117.

Filippov, S., Hrubýč, M., Konák, C. r., Macková, H., Spírkvá, M. and Stepánek, P. (2008) 'Novel pH-responsive nanoparticles', *Langmuir*, **24**, 9295–9301.

Förch, R., Chifen, A. N., Bousquet, A., Khor, H. L., Jungblut, M., Chu, L. Q., Zhang, Z., Osey-Mensah, I., Sinner, E. K. and Knoll, W. (2007) 'Recent and expected roles of plasma-polymerized films for biomedical applications', *Chemical Vapor Deposition*, **13**, 280–294.

Frank, S. (2004) 'Self-assembled monolayers: From 'simple' model systems to biofunctionalized interfaces', *Journal of Physics: Condensed Matter*, **16**, R881.

Friedrich, J. (2011) 'Mechanisms of plasma polymerization – Reviewed from a chemical point of view', *Plasma Processes and Polymers*, **8**, 783–802.

Fu, G.-Q., Yu, H. and Zhu, J. (2008) 'Imprinting effect of protein-imprinted polymers composed of chitosan and polyacrylamide: A re-examination', *Biomaterials*, **29**, 2138–2142.

Gall, K., Yakacki, C. M., Liu, Y., Shandas, R., Willett, N. and Anseth, K. S. (2005) 'Thermomechanics of the shape memory effect in polymers for biomedical applications', *Journal of Biomedical Materials Research Part A*, **73A**, 339–348.

Gan, D. and Lyon, L. A. (2003) 'Fluorescence nonradiative energy transfer analysis of crosslinker heterogeneity in core-shell hydrogel nanoparticles', *Analytica Chimica Acta*, **496**, 53–63.

Gao, Y., Xu, S., Wu, R., Wang, J. and Wei, J. (2008) 'Preparation and characteristic of electric stimuli responsive hydrogel composed of polyvinyl alcohol/poly (sodium maleate-co-sodium acrylate)', *Journal of Applied Polymer Science*, **107**, 391–395.

Geiger, E. J., Pisano, A. P. and Svec, F. (2010) 'A polymer-based microfluidic platform featuring on-chip actuated hydrogel valves for disposable applications', *Journal of Microelectromechanical Systems*, **19**, 944–950.

Golden, A. L., Battrell, C. F., Pennell, S., Hoffman, A. S., J. Lai, J. and Stayton, P. S. (2010) 'Simple fluidic system for purifying and concentrating diagnostic biomarkers using stimuli-responsive antibody conjugates and membranes', *Bioconjugate Chemistry*, **21**, 1820–1826.

Gupta, M. N. and Mattiasson, B. (2006), 'Unique applications of immobilized proteins in bioanalytical systems', *Methods of Biochemical Analysis*, John Wiley & Sons, Inc., Hoboken, NJ, USA, 1–34.

Hall, D. B., Underhill, P. and Torkelson, J. M. (1998) 'Spin coating of thin and ultra-thin polymer films', *Polymer Engineering & Science*, **38**, 2039–2045.

Hara, Y. and Yoshida, R. (2008) 'Self-oscillating polymer fueled by organic acid', *The Journal of Physical Chemistry B*, **112**, 8427–8429.

Herber, S., Bomer, J., Olthuis, W., Bergveld, P. and Berg, A. (2005a) 'A miniaturized carbon dioxide gas sensor based on sensing of pH-sensitive hydrogel swelling with a pressure sensor', *Biomedical Microdevices*, **7**, 197–204.

Herber, S., Bomer, J., Olthuis, W., Bergveld, P. and Van Den Berg, A. (2005b). A micro CO_2 gas sensor based on sensing of pH-sensitive hydrogel swelling by means of a pressure sensor, *IEEE*, **3C1.4**, 1146–1149.

Herber, S., Olthuis, W. and Bergveld, P. (2003) 'A swelling hydrogel-based P_{CO2} sensor', *Sensors and Actuators B: Chemical*, **91**, 378–382.

Herber, S., Olthuis, W., Bergveld, P. and van den Berg, A. (2004) 'Exploitation of a pH-sensitive hydrogel disk for CO_2 detection', *Sensors and Actuators B: Chemical*, **103**, 284–289.

Hermann, T. and Patel, D. J. (2000) 'Adaptive recognition by nucleic acid aptamers', *Science*, **287**, 820–825.

Hillberg, A. L., Brain, K. R. and Allender, C. J. (2005) 'Molecular imprinted polymer sensors: Implications for therapeutics', *Advanced Drug Delivery Reviews*, **57**, 1875–1889.

Hitchings, A. W. (2012) 'Monitoring drug therapy', *Medicine*, **40**, 376–381.

Hoare, T. and Pelton, R. (2008) 'Charge-switching, amphoteric glucose-responsive microgels with physiological swelling activity', *Biomacromolecules*, **9**, 733–740.

Hoffman, A. S. (1987) 'Applications of thermally reversible polymers and hydrogels in therapeutics and diagnostics', *Journal of Controlled Release*, **6**, 297–305.

Hoffman, A. S. (2000) 'Bioconjugates of intelligent polymers and recognition proteins for use in diagnostics and affinity separations', *Clinical Chemistry*, **46**, 1478–1486.

Hoffman, A. S. and Stayton, P. S. (2004) Bioconjugates of smart polymers and proteins: Synthesis and applications. *Macromolecular Symposia*, **207**, 139–152.

Hoffman, A. S. and Stayton, P. S. (2007) 'Conjugates of stimuli-responsive polymers and proteins', *Progress in Polymer Science*, **32**, 922–932.

Hoffman, A. S., Stayton, P. S., Bulmus, V., Chen, G., Chen, J., Cheung, C., Chilkoti, A., Ding, Z., Dong, L., Fong, R., Lackey, C. A., Long, C. J., Miura, M., Morris, J. E., Murthy, N., Nabeshima, Y., Park, T. G., Press, O. W., Shimoboji, T., Shoemaker, S., Yang, H. J., Monji, N., Nowinski, R. C., Cole, C. A., Priest, J. H., Harris, J. M., Nakamae, K., Nishino, T. and Miyata, T. (2000) 'Really smart bioconjugates of smart polymers and receptor proteins', *Journal of Biomedical Materials Research*, **52**, 577–586.

Hook, A. L., Voelcker, N. H. and Thissen, H. (2009) 'Patterned and switchable surfaces for biomolecular manipulation', *Acta Biomaterialia*, **5**, 2350–2370.

Hu, F. Q., Wei, L., Zhou, Z., Ran, Y. L., Li, Z. and Gao, M. Y. (2006) 'Preparation of biocompatible magnetite nanocrystals for in vivo magnetic resonance detection of cancer', *Advanced Materials*, **18**, 2553–2556.

Hu, J. (2010) 'Responsive polymers for detection and sensing applications: Current status and future developments', *Macromolecules*, **43**, 8315–8330.

Hua, Z., Chen, Z., Li, Y. and Zhao, M. (2008) 'Thermosensitive and salt-sensitive molecularly imprinted hydrogel for bovine serum albumin', *Langmuir*, **24**, 5773–5780.

Huang, C.-J., Chen, Y.-H., Wang, C.-H., Chou, T.-C. and Lee, G.-B. (2007) 'Integrated microfluidic systems for automatic glucose sensing and insulin injection', *Sensors and Actuators B: Chemical*, **122**, 461–468.

Huang, S. H. and Juang, R. S. (2011) 'Biochemical and biomedical applications of multifunctional magnetic nanoparticles: A review', *Journal of Nanoparticle Research*, **13**, 4411–4430.

Huang, W. M. (2010) 'Thermo-moisture responsive polyurethane shape memory polymer for biomedical devices', *The Open Medical Devices Journal*, **2**, 11–19.

Huang, W. M., Song, C. L., Fu, Y. Q., Wang, C. C., Zhao, Y., Purnawali, H., Lu, H. B., Tang, C., Ding, Z. and Zhang, J. L. (2012) 'Shaping tissue with shape memory materials', *Advanced Drug Delivery Reviews*, **65**(4), 515–535.

Huang, W. M., Yang, B., Zhao, Y. and Ding, Z. (2010a) 'Thermo-moisture responsive polyurethane shape-memory polymer and composites: A review', *Journal of Materials Chemistry*, **20**, 3367–3381.

Huang, X., Li, S., Schultz, J. S., Wang, Q. and Lin, Q. (2010b) 'A dielectric affinity microbiosensor', *Applied Physics Letters*, **96**, 033701–033701–033703.

Hyun Kim, J., Jin Kang, T. and Yu, W.-R. (2010) 'Simulation of mechanical behavior of temperature-responsive braided stents made of shape memory polyurethanes', *Journal of Biomechanics*, **43**, 632–643.

Immoos, C. E., Lee, S. J. and Grinstaff, M. W. (2004) 'DNA-Peg-DNA triblock macromolecules for reagentless DNA detection', *Journal of the American Chemical Society*, **126**, 10814–10815.

Ito, A., Kuga, Y., Honda, H., Kikkawa, H., Horiuchi, A., Watanabe, Y. and Kobayashi, T. (2004) 'Magnetite nanoparticle-loaded anti-Her2 immunoliposomes for combination of antibody therapy with hyperthermia', *Cancer Letters*, **212**, 167–175.

Jagur-Grodzinski, J. (2010) 'Polymeric gels and hydrogels for biomedical and pharmaceutical applications', *Polymers for Advanced Technologies*, **21**, 27–47.

Jegadesan, S., Advincula, R. C. and Valiyaveettil, S. (2005) 'Nanolithographic electropolymerization of a precursor polymer film to form conducting nanopatterns', *Advanced Materials*, **17**, 1282–1285.

Jeong, B. and Gutowska, A. (2002) 'Lessons from nature: Stimuli-responsive polymers and their biomedical applications', *Trends in Biotechnology*, **20**, 305–311.

Jiang, X. and Zhao, B. (2008) 'Tuning micellization and dissociation transitions of thermo- and pH-sensitive poly(ethylene oxide)-B-poly(methoxydi(ethylene glycol) methacrylate-co-methacrylic acid) in aqueous solution by combining temperature and ph triggers', *Macromolecules*, **41**, 9366–9375.

Jones, C. D. and Lyon, L. A. (2000) 'Synthesis and characterization of multiresponsive core-shell microgels', *Macromolecules*, **33**, 8301–8306.

Jun, Y.-w., Huh, Y.-M., Choi, J.-s., Lee, J.-H., Song, H.-T., KimKim, Yoon, S., Kim, K.-S., Shin, J.-S., Suh, J.-S. and Cheon, J. (2005) 'Nanoscale size effect of magnetic nanocrystals and their utilization for cancer diagnosis via magnetic resonance imaging', *Journal of the American Chemical Society*, **127**, 5732–5733.

Jung, Y. and Cho, J. (2010) 'Application of shape memory polyurethane in orthodontic', *Journal of Materials Science: Materials in Medicine*, **21**, 2881–2886.

Kahan, B. D., Keown, P., Levy, G. A. and Johnston, A. (2002) 'Therapeutic drug monitoring of immunosuppressant drugs in clinical practice', *Clinical Therapeutics*, **24**, 330–350.

Kang, D., Zuo, X., Yang, R., Xia, F., Plaxco, K. W. and White, R. J. (2009) 'Comparing the properties of electrochemical-based DNA sensors employing different redox tags', *Analytical Chemistry*, **81**, 9109–9113.

Katz, J. S. and Burdick, J. A. (2010) 'Light-responsive biomaterials: Development and applications', *Macromolecular Bioscience*, **10**, 339–348.

Kang, S. I. and Bae, Y. H. (2003) 'A sulfonamide based glucose-responsive hydrogel with covalently immobilized glucose oxidase and catalase', *Journal of Controlled Release*, **86**, 115–121.

Kesselman, L. R. B., Shinwary, S., Selvaganapathy, P. R. and Hoare, T. (2012) 'Synthesis of monodisperse, covalently cross-linked, degradable 'smart' microgels using microfluidics', *Small*, **8**, 1092–1098.

Kim, S. J., Yoon, S. G., Lee, S. M., Lee, S. H. and Kim, S. I. (2004) 'Electrical sensitivity behavior of a hydrogel composed of polymethacrylic acid/poly(vinyl alcohol)', *Journal of Applied Polymer Science*, **91**, 3613–3617.

Kirsebom, H., Galaev, I. Y. and Mattiasson, B. (2010) 'Stimuli-responsive polymers in the 21st century: Elaborated architecture to achieve high sensitivity, fast response, and robust behavior', *Journal of Polymer Science, Part B: Polymer Physics*, **49**, 173–178.

Knight, P. T., Lee, K. M., Qin, H. and Mather, P. T. (2008) 'Biodegradable thermoplastic polyurethanes incorporating polyhedral oligosilsesquioxane', *Biomacromolecules*, **9**, 2458–2467.

Knight, P. T., Lee, K. M., Chung, T. and Mather, P. T. (2009) 'Plga-poss end-linked networks with tailored degradation and shape memory behavior', *Macromolecules*, **42**, 6596–6604.

Koňák, Č., Pánek, J. and Hrubý, M. (2007) 'Thermoresponsive polymeric nanoparticles stabilized by surfactants', *Colloid & Polymer Science*, **285**, 1433–1439.

Kopeček, J. (2007) 'Hydrogel biomaterials: A smart future?', *Biomaterials*, **28**, 5185–5192.

Kopeček, J. and Yang, J. (2007) 'Hydrogels as smart biomaterials', *Polymer International*, **56**, 1078–1098.

Koppolu, B., Rahimi, M., Nattama, S., Wadajkar, A. and Nguyen, K. T. (2010) 'Development of multiple-layer polymeric particles for targeted and controlled drug delivery', *Nanomedicine: Nanotechnology, Biology and Medicine*, **6**, 355–361.

Kwan, R. C. H., Hon, P. Y. T., Mak, K. K. W. and Renneberg, R. (2004a) 'Amperometric determination of lactate with novel trienzyme/poly(carbamoyl) sulfonate hydrogel-based sensor', *Biosensors and Bioelectronics*, **19**, 1745–1752.

Kwan, R. C. H., Hon, P. Y. T. and Renneberg, R. (2004b) 'Amperometric biosensor for rapid determination of alanine', *Analytica Chimica Acta*, **523**, 81–88.

Kwan, R. C. H., Hon, P. Y. T. and Renneberg, R. (2005) 'Amperometric determination of ammonium with bienzyme/poly(carbamoyl) sulfonate hydrogel-based biosensor', *Sensors and Actuators B: Chemical*, **107**, 616–622.

Kwan, R. C. H., Hon, P. Y. T., Mak, W. C., Law, L. Y., Hu, J. and Renneberg, R. (2006) 'Biosensor for rapid determination of 3-hydroxybutyrate using bienzyme system', *Biosensors and Bioelectronics*, **21**, 1101–1106.

Lahann, J., Mitragotri, S., Tran, T.-N., Kaido, H., Sundaram, J., Choi, I. S., Hoffer, S., Somorjai, G. A. and Langer, R. (2003) 'A reversibly switching surface', *Science*, **299**, 371–374.

Lai, J. J., Hoffman, J. M., Ebara, M., Hoffman, A. S., EstournÃ¨s, C., Wattiaux, A. and Stayton, P. S. (2007) 'Dual magnetic-/temperature-responsive nanoparticles for microfluidic separations and assays', *Langmuir*, **23**, 7385–7391.

Lai, J. J., Nelson, K. E., Nash, M. A., Hoffman, A. S., Yager, P. and Stayton, P. S. (2009) 'Dynamic bioprocessing and microfluidic transport control with smart magnetic nanoparticles in laminar-flow devices', *Lab on a Chip*, **9**, 1997–2002.

Langer, R. and Tirrell, D. A. (2004) 'Designing materials for biology and medicine', *Nature*, **428**, 487–492.

Lapeyre, V. r., Ancla, C., Catargi, B. and Ravaine, V. r. (2008) 'Glucose-responsive microgels with a core-shell structure', *Journal of Colloid and Interface Science*, **327**, 316–323.

Lee, H., Lee, E., Kim, D. K., Jang, N. K., Jeong, Y. Y. and Jon, S. (2006) 'Antibiofouling polymer-coated superparamagnetic iron oxide nanoparticles as potential magnetic resonance contrast agents for in vivo cancer imaging', *Journal of the American Chemical Society*, **128**, 7383–7389.

Lendlein, A. and Langer, R. (2002) 'Biodegradable, elastic shape-memory polymers for potential biomedical applications', *Science*, **296**, 1673–1676.

Lendlein, A. and Langer, R. (2004) Self-expanding device for the gastrointestinal or urogenital area. Canadian patent application CA 2516285.

Leng, J., Lu, H., Liu, Y., Huang, W. M. and Du, S. (2009) 'Shape-memory polymers – A class of novel smart materials', *MRS Bulletin*, **34**, 848–855.

Liu, F., Song, S. C., Mix, D., Baudyš, M. and Kim, S. W. (1997) 'Glucose-induced release of glycosylpoly(ethylene glycol) insulin bound to a soluble conjugate of concanavalin a', *Bioconjugate Chemistry*, **8**, 664–672.

Lu, Z.-R., Kopečková, P. and Kopeček, J. (2003) 'Antigen responsive hydrogels based on polymerizable antibody fab′ fragment', *Macromolecular Bioscience*, **3**, 296–300.

Lutz, J.-F. and Börner, H. G. (2008) 'Modern trends in polymer bioconjugates design', *Progress in Polymer Science*, **33**, 1–39.

Maeda, S., Hara, Y., Yoshida, R. and Hashimoto, S. (2008) 'Control of the dynamic motion of a gel actuator driven by the Belousov-Zhabotinsky reaction', *Macromolecular Rapid Communications*, **29**, 401–405.

Magis-Escurra, C., van den Boogaard, J., Ijdema, D., Boeree, M. and Aarnoutse, R. (2012) 'Therapeutic drug monitoring in the treatment of tuberculosis patients', *Pulmonary Pharmacology and Therapeutics*, **25**, 83–86.

Maitland, D. J., Lee, A., Shumann, D. and Da Silva, L., (1998) Shape memory polymer gripper release system with sensing of target release. International patent application PCT/US1999/015101.

Maitland, D. J., Metzger, M. F., Schumann, D., Lee, A. and Wilson, T. S. (2002) 'Photothermal properties of shape memory polymer micro-actuators for treating stroke', *Lasers in Surgery and Medicine*, **30**, 1–11.

Marco, D. (2006). Biodegradable self-inflating intragastric implants and method of curbing appetite by the same. International patent application PCT/IL2006/000276.

Mather, P. T., Luo, X. and Rousseau, I. A. (2009) 'Shape memory polymer research', *Annual Review of Materials Research*, **39**, 445–471.

McKee, P. J. W., Percy-Robb, I. and Brodie, M. J. (1992) 'Therapeutic drug monitoring improves seizure control and reduces anticonvulsant side-effects in patients with refractory epilepsy', *Seizure*, **1**, 275–279.

Medeiros, S. F., Santos, A. M., Fessi, H. and Elaissari, A. (2010) 'Stimuli-responsive magnetic particles for biomedical applications', *International Journal of Pharmaceutics*, **403**, 139–161.

Mendes, P. M. (2008) 'Stimuli-responsive surfaces for bio-applications', *Chemical Society Reviews*, **37**, 2512–2529.

Mendes, P., Yeung, C. and Preece, J. (2007) 'Bio-nanopatterning of surfaces', *Nanoscale Research Letters*, **2**, 373–384.

Meng, Q. and Hu, J. (2009) 'A review of shape memory polymer composites and blends', *Composites Part A: Applied Science and Manufacturing*, **40**, 1661–1672.

Metzger, M. F., Wilson, T. S., Schumann, D., Matthews, D. L. and Maitland, D. J. (2002) 'Mechanical properties of mechanical actuator for treating ischemic stroke', *Biomedical Microdevices*, **4**, 89–96.

Minko, S., Ionov, L., Sydorenko, A., Houbenov, N., Stamm, M., Zdyrko, B., Klep, V. and Luzinov, I. (2005) 'Gradient stimuli-responsive polymer grafted layers', *Stimuli-Responsive Polymeric Films and Coatings*, American Chemical Society, USA. 68–83.

Miyata, T., Asami, N. and Uragami, T. (1999a) 'Preparation of an antigen-sensitive hydrogel using antigen-antibody bindings', *Macromolecules*, **32**, 2082–2084.

Miyata, T., Asami, N. and Uragami, T. (1999b) 'A reversibly antigen-responsive hydrogel', *Nature*, **399**, 766–769.

Miyata, T., Jige, M., Nakaminami, T. and Uragami, T. (2006) 'Tumor marker-responsive behavior of gels prepared by biomolecular imprinting', *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 1190–1193.

Motornov, M., Roiter, Y., Tokarev, I. and Minko, S. (2010) 'Stimuli-responsive nanoparticles, nanogels and capsules for integrated multifunctional intelligent systems', *Progress in Polymer Science*, **35**, 174–211.

Murase, Y., Maeda, S., Hashimoto, S. and Yoshida, R. (2008) 'Design of a mass transport surface utilizing peristaltic motion of a self-oscillating gel', *Langmuir*, **25**, 483–489.

Na, H. B., Song, I. C. and Hyeon, T. (2009) 'Inorganic nanoparticles for MRI contrast agents', *Advanced Materials*, **21**, 2133–2148.

Nakasima, A., Hu, J. R., Ichinose, M. and Shimada, H. (1991) 'Potential application of shape memory plastic as elastic material in clinical orthodontics', *The European Journal of Orthodontics*, **13**, 179–186.

Nash, M. A., Lai, J. J. *et al.* (2010) 'Smart' diblock copolymers as templates for magnetic-core gold-shell nanoparticle synthesis', *Nano Letters*, **10**, 85–91.

Nash, M. A., Lai, J. J., Hoffman, A. S., Yager, P. and Stayton, P. S. (2010) 'Mixed stimuli-responsive magnetic and gold nanoparticle system for rapid purification, enrichment, and detection of biomarkers', *Bioconjugate Chemistry*, **21**, 2197–2204.

Nawrat, Z. (2008) 'Review of research in cardiovascular devices', In: P. Verdonck (ed.), *Advances in Biomedical Engineering*. Elsevier Science, St. Louis, 1–59.

Ng, A. H. C., Uddayasankar, U. and Wheeler, A. R. (2010) 'Immunoassays in microfluidic systems', *Analytical and Bioanalytical Chemistry*, **397**, 991–1007.

Nuzzo, R. and Allara, D. (1983) 'Adsorption of bifunctional organic disulfides on gold surfaces', *Journal of the American Chemical Society*, **105**, 4481–4483.

Ogiso, M., Minoura, N., Shinbo, T. and Shimizu, T. (2006) 'Detection of a specific DNA sequence by electrophoresis through a molecularly imprinted polymer', *Biomaterials*, **27**, 4177–4182.

Ohnishi, N., Satoh, W., Morimoto, K., Fukuda, J. and Suzuki, H. (2010) 'Automatic electrochemical sequential processing in a microsystem for urea detection', *Sensors and Actuators B: Chemical*, **144**, 146–152.

Olivier, A., Meyer, F., Raquez, J.-M., Damman, P. and Dubois, P. (2011) 'Surface-initiated controlled polymerization as a convenient method for designing functional polymer brushes: From self-assembled monolayers to patterned surfaces', *Progress in Polymer Science*, **37**, 157–181.

Onclin, S., Ravoo, B. J. and Reinhoudt, D. N. (2005) 'Engineering silicon oxide surfaces using self-assembled monolayers', *Angewandte Chemie International Edition*, **44**, 6282–6304.

Ortega, J. M. and Small, W. (2007) 'A shape memory polymer dialysis needle adapter for the reduction of hemodynamic stress within arteriovenous grafts', *IEEE Transactions on Biomedical Engineering*, **54**, 1722–1724.

Pennadam, S. S. (2004) 'Protein-polymer nano-machines. Towards synthetic control of biological processes', *Journal of Nanobiotechnology*, **2**, 8.

Pierce, B. F., Brown, A. H. and Sheares, V. V. (2008) 'Thermoplastic poly(ester urethane)s with novel soft segments', *Macromolecules*, **41**, 3866–3873.

Podual, K., Doyle Iii, F. J. and Peppas, N. A. (2000) 'Glucose-sensitivity of glucose oxidase-containing cationic copolymer hydrogels having poly(ethylene glycol) grafts', *Journal of Controlled Release*, **67**, 9–17.

Ponmozhi, J., Frias, C., Marques, T. and Frazão, O. (2012) 'Smart sensors/actuators for biomedical applications: Review', *Measurement*, **45**, 1675–1688.

Purushotham, S. and Ramanujan, R. V. (2010) 'Thermoresponsive magnetic composite nanomaterials for multimodal cancer therapy', *Acta Biomaterialia*, **6**, 502–510.

Qing, G. and Sun, T. (2011) 'Chirality-triggered wettability switching on a smart polymer surface', *Advanced Materials*, **23**, 1615–1620.

Radt, B., Smith, T. A. and Caruso, F. (2004) 'Optically addressable nanostructured capsules', *Advanced Materials*, **16**, 2184–2189.

Ratna, D. and Karger-Kocsis, J. (2008) 'Recent advances in shape memory polymers and composites: A review', *Journal of Materials Science*, **43**, 254–269.

Ravichandran, R., Sundarrajan, S., Venugopal, J. R., Mukherjee, S. and Ramakrishna, S. (2012) 'Advances in polymeric systems for tissue engineering and biomedical applications', *Macromolecular Bioscience*, **12**, 286–311.

Ricci, F., Lai, R. Y. and Plaxco, K. W. (2007) 'Linear, redox modified DNA probes as electrochemical DNA sensors', *Chemical Communications*, 3768–3770. DOI: 10.1039/b708882e.

Rieger, J., Grazon, C., Charleux, B., Alaimo, D. and Jérôme, C. (2009) 'Pegylated thermally responsive block copolymer micelles and nanogels via in situ raft aqueous dispersion polymerization', *Journal of Polymer Science Part A: Polymer Chemistry*, **47**, 2373–2390.

Rivet, C., Lee, H., Hirsch, A., Hamilton, S. and Lu, H. (2010) 'Microfluidics for medical diagnostics and biosensors', *Chemical Engineering Science*, **66**, 1490–1507.

Rodríguez-Hernández, J., Chécot, F., Gnanou, Y. and Lecommandoux, S. (2005) 'Toward 'smart' nano-objects by self-assembly of block copolymers in solution', *Progress in Polymer Science*, **30**, 691–724.

Rogers, J. A. and Nuzzo, R. G. (2005) 'Recent progress in soft lithography', *Materials Today*, **8**, 50–56.

Roy, D., Cambre, J. N. and Sumerlin, B. S. (2010) 'Future perspectives and recent advances in stimuli-responsive materials', *Progress in Polymer Science (Oxford)*, **35**, 278–301.

Roy, I. and Gupta, M. N. (2003) 'Smart polymeric materials: Emerging biochemical applications', *Chemistry & Biology*, **10**, 1161–1171.

Samchenko, Y., Ulberg, Z. and Korotych, O. (2011) 'Multipurpose smart hydrogel systems', *Advances in Colloid and Interface Science*, **168**, 247–262.

Sarmento, B., Ribeiro, A. n., Veiga, F., Ferreira, D. and Neufeld, R. (2007) 'Oral bioavailability of insulin contained in polysaccharide nanoparticles', *Biomacromolecules*, **8**, 3054–3060.

Schreiber, F. (2000) 'Structure and growth of self-assembling monolayers', *Progress in Surface Science*, **65**, 151–257.

Seiffert, S. and Weitz, D. A. (2010) 'Microfluidic fabrication of smart microgels from macromolecular precursors', *Polymer*, **51**, 5883–5889.

Seong, H., Lee, H. B. and Park, K. (2002) 'Glucose binding to molecularly imprinted polymers', *Journal of Biomaterials Science, Polymer Edition*, **13**, 637–649.

Sharp, A. A., Panchawagh, H. V., Ortega, A., Artale, R., Richardson-Burns, S., Finch, D. S., Gall, K., Mahajan, R. L. and Restrepo, D. (2006) 'Toward a self-deploying shape memory polymer neuronal electrode', *Journal of Neural Engineering*, **3**, L23.

Shaw, L. M., Holt, D. W., Keown, P., Venkataramanan, R. and Yatscoff, R. W. (1999) 'Current opinions on therapeutic drug monitoring of immunosuppressive drugs', *Clinical Therapeutics*, **21**, 1632–1652.

Shimoboji, T., Larenas, E., Fowler, T., Kulkarni, S., Hoffman, A. S. and Stayton, P. S. (2002) 'Photoresponsive polymer-enzyme switches', *Proceedings of the National Academy of Sciences*, **99**, 16592–16596.

Shinkai, M. (2002) 'Functional magnetic particles for medical application', *Journal of Bioscience and Bioengineering*, **94**, 606–613.

Shtanko, N. I., Lequieu, W., Goethals, E. J. and Du Prez, F. E. (2003) 'pH- and thermo-responsive properties of poly(N-vinylcaprolactam-co-acrylic acid) copolymers', *Polymer International*, **52**, 1605–1610.

Sia, S. K. and Kricka, L. J. (2008) 'Microfluidics and point-of-care testing', *Lab on a Chip*, **8**, 1982–1983.

Small, I. V. W., Singhal, P., Wilson, T. S. and Maitland, D. J. (2010) 'Biomedical applications of thermally activated shape memory polymers', *Journal of Materials Chemistry*, **20**, 3356–3366.

Smela, E. (2003) 'Conjugated polymer actuators for biomedical applications', *Advanced Materials*, **15**, 481–494.

Smith, R. K., Lewis, P. A. and Weiss, P. S. (2004) 'Patterning self-assembled monolayers', *Progress in Surface Science*, **75**, 1–68.

Sokolowski, W. (2010) 'Shape memory polymer foams for biomedical devices', *The Open Medical Devices Journal*, **2**, 20–23.

Sokolowski, W., Metcalfe, A., Hayashi, S., Yahia, L. and Raymond, J. (2007) 'Medical applications of shape memory polymers', *Biomedical Materials*, **2**, S23–27.

Soper, S. A., Brown, K., Ellington, A., Frazier, B., Garcia-Manero, G., Gau, V., Gutman, S. I., Hayes, D. F., Korte, B., Landers, J. L., Larson, D., Ligler, F., Majumdar, A., Mascini, M., Nolte, D., Rosenzweig, Z., Wang, J. and Wilson, D. (2006) 'Point-of-care biosensor systems for cancer diagnostics/prognostics', *Biosensors and Bioelectronics*, **21**, 1932–1942.

Stuart, M. A. C., Huck, W. T. S., Genzer, J., Müller, M., Ober, C., Stamm, M., Sukhorukov, G. B., Szleifer, I., Tsukruk, V. V., Urban, M., Winnik, F., Zauscher, S., Luzinov, I. and Minko, S. (2010) 'Emerging applications of stimuli-responsive polymer materials', *Nature Materials*, **9**, 101–113.

Such, G. K., Tjipto, E., Postma, A., Johnston, A. P. R. and Caruso, F. (2007) 'Ultrathin, responsive polymer click capsules', *Nano Letters*, **7**, 1706–1710.

Sun, L., Huang, W. M., Ding, Z., Zhao, Y., Wang, C. C., Purnawali, H. and Tang, C. (2012) 'Stimulus-responsive shape memory materials: A review', *Materials and Design*, **33**, 577–640.

Szarowski, D. H., Andersen, M. D., Retterer, S., Spence, A. J., Isaacson, M., Craighead, H. G., Turner, J. N. and Shain, W. (2003) 'Brain responses to micro-machined silicon devices', *Brain Research*, **983**, 23–35.

Tagit, O. (2009) 'Temperature-modulated quenching of quantum dots covalently coupled to chain ends of poly(N-isopropyl acrylamide) brushes on gold', *Nanotechnology*, **20**, 185501.

Takashima, K., Rossiter, J. and Mukai, T. (2010) 'Mckibben artificial muscle using shape-memory polymer', *Sensors and Actuators A: Physical*, **164**, 116–124.

Takeuchi, T. and Hishiya, T. (2008) 'Molecular imprinting of proteins emerging as a tool for protein recognition', *Organic & Biomolecular Chemistry*, **6**, 2459–2467.

Tanna, S. (2006) 'Glucose-responsive UV polymerised dextran–concanavalin A acrylic derivatised mixtures for closed-loop insulin delivery', *Biomaterials*, **27**, 1586.

Tasci, T. O., Vargel, I., Arat, A., Guzel, E., Korkusuz, P. and Atalar, E (2009) 'Focused Rf hyperthermia using magnetic fluids', *Medical Physics*, **36**, 1906–1912.

Theato, P. (2008) 'Synthesis of well-defined polymeric activated esters', *Journal of Polymer Science Part A: Polymer Chemistry*, **46**, 6677–6687.

Tierney, M. J., Kim, H. L., Burns, M. D., Tamada, J. A. and Potts, R. O. (2000) 'Electroanalysis of glucose in transcutaneously extracted samples', *Electroanalysis*, **12**, 666–671.

Tokarev, I. and Minko, S. (2009a) 'Multiresponsive, hierarchically structured membranes: New, challenging, biomimetic materials for biosensors, controlled release, biochemical gates, and nanoreactors', *Advanced Materials*, **21**, 241–247.

Tokarev, I. and Minko, S. (2009b) 'Stimuli-responsive hydrogel thin films', *Soft Matter*, **5**, 511–524.

Tokarev, I., Orlov, M., Katz, E. and Minko, S. (2007) 'An electrochemical gate based on a stimuli-responsive membrane associated with an electrode surface', *The Journal of Physical Chemistry B*, **111**, 12141–12145.

Tokarev, I., Orlov, M. and Minko, S. (2006) 'Responsive polyelectrolyte gel membranes', *Advanced Materials*, **18**, 2458–2460.

Tokarev, I., Tokareva, I. and Minko, S. (2008) 'Gold-nanoparticle-enhanced plasmonic effects in a responsive polymer gel', *Advanced Materials*, **20**, 2730–2734.

Tokareva, I., Minko, S., Fendler, J. H. and Hutter, E. (2004) 'Nanosensors based on responsive polymer brushes and gold nanoparticle enhanced transmission surface plasmon resonance spectroscopy', *Journal of the American Chemical Society*, **126**, 15950–15951.

Tu, H., Hong, L., Anthony, S. M., Braun, P. V. and Granick, S. (2007) 'Brush-sheathed particles diffusing at brush-coated surfaces in the thermally responsive PNIPAAM system', *Langmuir*, **23**, 2322–2325.

Uhlmann, P., Ionov, L., Houbenov, N., Nitschke, M., Grundke, K., Motornov, M., Minko, S. and Stamm, M. (2006) 'Surface functionalization by smart coatings: Stimuli-responsive binary polymer brushes', *Progress in Organic Coatings*, **55**, 168–174.

Ulman, A. (1996) 'Formation and structure of self-assembled monolayers', *Chemical Reviews*, **96**, 1533–1554.

Vaddiraju, S., Tomazos, I., Burgess, D. J., Jain, F. C. and Papadimitrakopoulos, F. (2010) 'Emerging synergy between nanotechnology and implantable biosensors: A review', *Biosensors and Bioelectronics*, **25**, 1553–1565.

Wache, H. M., Tartakowska, D. J., Hentrich, A. and Wagner, M. H. (2003) 'Development of a polymer stent with shape memory effect as a drug delivery system', *Journal of Materials Science: Materials in Medicine*, **14**, 109–112.

Wandera, D., Wickramasinghe, S. R. and Husson, S. M.. (2010) 'Stimuli-responsive membranes', *Journal of Membrane Science*, **357**, 6–35.

Wang, B., Liu, M.-Z., Liang, R., Ding, S.-L., Chen, Z.-B., Chen, S.-L. and Jin, S.-P. (2008) 'Mmtca recognition by molecular imprinting in interpenetrating polymer network hydrogels based on poly(acrylic acid) and poly(vinyl alcohol)', *Macromolecular Bioscience*, **8**, 417–425.

Wang, J. (2001) 'Glucose biosensors: 40 years of advances and challenges', *Electroanalysis*, **13**, 983.

Wilson, G. S. and Gifford, R. (2005) 'Biosensors for real-time in vivo measurements', *Biosensors and Bioelectronics*, **20**, 2388–2403.

Wong, J. E., Gaharwar, A. K., Müller-Schulte, D., Bahadur, D. and Richtering, W. (2008) 'Dual-stimuli responsive pnipam microgel achieved via layer-by-layer assembly: Magnetic and thermoresponsive', *Journal of Colloid and Interface Science*, **324**, 47–54.

Wust, P. (2002) 'Hyperthermia in combined treatment of cancer', *Lancet Oncology*, **3**, 487–497.

Wust, P., Hildebrandt, B., Sreenivasa, G., Rau, B., Gellermann, J., Riess, H., Felix, R. and Schlag, P. M. (2002) 'Hyperthermia in combined treatment of cancer', *The Lancet Oncology*, **3**, 487–497.

Xu, C., Wu, T., Drain, C. M., Batteas, J. D., Fasolka, M. J. and Beers, K. L. (2006) 'Effect of block length on solvent response of block copolymer brushes: Combinatorial study with block copolymer brush gradients', *Macromolecules*, **39**, 3359–3364.

Xue, L., Dai, S. and Li, Z. (2010) 'Biodegradable shape-memory block co-polymers for fast self-expandable stents', *Biomaterials*, **31**, 8132–8140.

Yakacki, C. M., Shandas, R., Lanning, C., Rech, B., Eckstein, A. and Gall, K. (2007) 'Unconstrained recovery characterization of shape-memory polymer networks for cardiovascular applications', *Biomaterials*, **28**, 2255–2263.

Yakacki, C. M., Willis, S., Luders, C. and Gall, K. (2008) 'Deformation limits in shape-memory polymers', *Advanced Engineering Materials*, **10**, 112–119.

Yang, L. and Liu, H. (2013) 'Stimuli-responsive magnetic particles and their applications in biomedical field', *Powder Technology*, **240**, 54–65.

Yanul, N. A., Kirsh, Y. E., Verbrugghe, S., Goethals, E. J. and Du Prez, F. E. (2001) 'Thermoresponsive properties of poly(N-vinylcaprolactam)-poly(ethylene oxide) aqueous systems: Solutions and block copolymer networks', *Macromolecular Chemistry and Physics*, **202**, 1700–1709.

Yu, Q. (2001) 'Responsive Biomimetic hydrogel valve for microfluidics', *Applied Physics Letters*, **78**, 2589.

Yusa, S.-i., Sugahara, M., Endo, T. and Morishima, Y. (2009) 'Preparation and characterization of a pH-responsive nanogel based on a photo-cross-linked micelle formed from block copolymers with controlled structure', *Langmuir*, **25**, 5258–5265.

Zhang, J. and Han, Y. (2010) 'Active and responsive polymer surfaces', *Chemical Society Reviews*, **39**, 676–693.

Zhang, K., Ma, J., Zhang, B., Zhao, S., Li, Y., Xu, Y., Yu, W. and Wang, J. (2007a) 'Synthesis of thermoresponsive silica nanoparticle/PNIPAM hybrids by aqueous surface-initiated atom transfer radical polymerization', *Materials Letters*, **61**, 949–952.

Zhang, R., Bowyer, A., Eisenthal, R. and Hubble, J. (2007b) 'A smart membrane based on an antigen-responsive hydrogel', *Biotechnology and Bioengineering*, **97**, 976–984.

Zhang, S.-B., Chu, L.-Y., Xu, D., Zhang, J., Ju, X.-J. and Xie, R. (2008) 'Poly(N-isopropylacrylamide)-based comb-type grafted hydrogel with rapid response to blood glucose concentration change at physiological temperature', *Polymers for Advanced Technologies*, **19**, 937–943.

Zhao, C., Nie, S., Tang, M. and Sun, S. (2011) 'Polymeric pH-sensitive membranes – A review', *Progress in Polymer Science*, **36**, 1499–1520.

Zheng, X., Zhou, S., Li, X. and Weng, J. (2006) 'Shape memory properties of poly(D,L-lactide)/hydroxyapatite composites', *Biomaterials*, **27**, 4288–4295.

Zhou, L., Yuan, J., Yuan, W., Sui, X., Wu, S., Li, Z.. and Shen, D. (2009) 'Synthesis, characterization, and controllable drug release of pH-sensitive hybrid magnetic nanoparticles', *Journal of Magnetism and Magnetic Materials*, **321**, 2799–2804.

Ziaie, B., Baldi, A., Lei, M., Gu, Y. and Siegel, R. A. (2004) 'Hard and soft micromachining for biomems: Review of techniques and examples of applications in microfluidics and drug delivery', *Advanced Drug Delivery Reviews*, **56**, 145–172.

13

Smart polymers for bioseparation and other biotechnological applications

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Abstract: The progress in development of a number of novel products using recombinant DNA technology and cell culturing, plus the demands for high product yield whilst preserving biological activity, require novel approaches for fast and cost-effective isolation and/or purification processes. Smart polymers (SPs) with their ability to undergo considerable changes in response to external stimuli make possible the development of novel technologies for isolation and purification. In this chapter the main applications of SPs in biotechnology and, in particular, in bioseparation, are discussed. Affinity precipitation, two-phase polymer separation, using SP membranes and SP chromatographic carriers are overviewed with a presentation of recent developments and discussion of future perspectives in these areas. Application of SP as catalysts is also discussed.

Key words: smart polymer, bioseparation, affinity precipitation, two-phase systems, membranes, chromatography, adsorbents, catalysts.

13.1 Introduction

Polymers which change in response to external stimuli are called intelligent polymers, stimuli-responsive polymers or smart polymers (SPs). In this chapter we summarise the current applications of SPs in the area of biotechnology. Progress in biotechnology has led to a development of a number of products using recombinant DNA technology and cell culturing. One of the essential steps in manufacturing is recovery and purification of the product. The cost of the final product is largely dependent on the cost of the separation and purification process. There is a constant need to develop fast and cost-effective isolation/purification processes with high product yield without losing the biological activity of the product.

All separation processes in general, and bioseparation processes in particular, include three stages:

- preferential partitioning of the target substance and impurities between two phases (liquid–liquid, liquid–gas or liquid–solid);
- mechanical separation of the phases (e.g. separation of the stationary and mobile phase in a chromatographic column or the separation of precipitate and liquid phase in a centrifuge);
- recovery of the target substance from the enriched phase.

SPs have the ability to respond non-linearly by exhibiting significant changes in their conformation at the microscopic level as a result of small changes in their environment such as pH, temperature or ionic strength, or the presence of specific trigger compounds. At the macroscopic level, these changes are translated to avalanche changes in the whole system such as phase separation or dramatic changes in interface properties.

The ability to undergo phase separation makes it possible to use SPs in affinity precipitation where the SP ensures the formation of a new phase. New phases include polymer precipitate enriched with a specifically bound target substance or aqueous two-phase systems where the phase-forming SP can be easily separated by precipitation from the target substance, thus facilitating its recovery. Alternatively, a change in the conformation of the SP grafted at the solid–liquid interface influences the interaction of the target substance present in the liquid phase with the solid phase for example, adsorption or permeation through the pores.

The application of SPs for purification of different substances was reviewed extensively in (Galaev and Mattiasson, 2002) followed recently by the second revised and extended edition (Galaev and Mattiasson, 2007). This chapter presents a brief overview of the fundamentals of SP applications in such purification techniques as:

- affinity precipitation
- aqueous polymer two-phase partitioning
- controlled permeation through membranes
- thermosensitive chromatography.

The emphasis is on recent developments in the field that have taken place in the last five years.

13.2 Smart polymers (SPs) for bioseparation: use in affinity precipitation

Precipitation is one of the common methods for the separation of the target molecules. Precipitation of proteins is based on changing their solubility by the addition of organic solvents or a large amount of salts. However, this method is not very selective as it is limited to the difference in the surface

properties of the proteins. Better selectivity could be achieved by using an affinity ligand or biorecognition ligand which reacts specifically with the target molecule. The ability of SPs to change their conformation as a result of small changes in the environment was used to facilitate the precipitation process and recovery of the target proteins. Attaching the affinity ligand to a SP makes the total separation process simpler, faster and more efficient (Kumar *et al.*, 2007).

In conventional affinity precipitation, SP with the affinity ligand first forms a complex with the target product. The phase separation of the complex is then triggered by changes in the environment, such as temperature or salt concentration. Under the effect of stimuli, the SPs change their conformation, become insoluble and precipitate in the complex with the target protein whilst all the impurities remain in solution. The two phases are separated mechanically and the protein is then recovered using one of two methods. The two methods are: eluting from the insoluble SP complex, or dissolution and dissociation of the protein from the SP, followed by re-precipitation of the polymer without protein.

Ideally a smart polymer for affinity precipitation should not interact with the affinity ligand or impurities to avoid non-specific precipitation. It should provide complete phase separation of the affinity complex and easy separation from the solution. To reduce the cost of the total purification process, the SP should be easily solubilised after the precipitate is formed and easily recovered with high yield to be reused in further cycles of precipitation-solubilisation. SP with the affinity ligand could be prepared by covalent linking of the affinity ligand to the polymer directly, through the spacer or alternatively by the copolymerisation of SP with functional co-monomer bearing the affinity ligand.

The affinity precipitation technique was mainly developed to separate proteins from a crude, unprocessed extract that would contain a complex mixture of proteins, cell debris, cell membranes, and particulate material. Thus the affinity ligand used should withstand these harsh conditions as well as the further steps of precipitation and recovery. There have been several successful applications of various ligands demonstrated (Gupta and Mattiasson, 1994) such as sugar ligands, triazine dyes and chelating groups charged with metal ions.

Application of affinity precipitation of proteins using SPs emerged in the late 1980s (Senstad and Mattiasson, 1989). A number of applications have been developed since then and reviewed in detail (Kumar *et al.*, 2007). In this chapter, the general overview of the method is given with demonstrations of some examples from recent research.

One of the most successful and well-known technologies in affinity purification is using metal chelate ligands for protein separation, particularly for proteins genetically modified with a polyhistidine affinity tag (His tag).

(Block *et al.*, 2009). The technology is based on selective interaction of the recombinant proteins with metal ions such as Cu(II) or Ni(II). Polydentate carboxy-containing ligands such as iminodiacetic acid (IDA) and nitrilotriacetic acid (NTA) are traditionally used as metal-chelating ligands in immobilised metal-affinity chromatography (IMAC). IDA and NTA coordinate well with metal ions leaving some coordination sites available for interaction with imidazole residues at the surface of protein molecules. For affinity precipitation monomers with these ligands are copolymerised with a smart polymer. This is a simpler and more cost-effective method for large-scale processing in comparison with chromatographic techniques, which require completely particulate-free solutions to prevent clogging of the column.

According to a standard protocol, the copolymer with metal-chelating groups is first charged with metal ions, allowing the protein to bind to a metal-loaded polymer where it is precipitated from solution at an increased temperature. The protein-polymer complexes are isolated, solubilised and the protein is then dissociated from the complex by using imidazole or ethylenediamine tetraacetic acid (EDTA). The SPs are recovered from the protein solution by secondary precipitation at elevated temperature and reused in the next precipitation cycle. The multiple reuse of the SP with an affinity ligand attached makes the whole process more cost effective.

The temperature and efficiency of SP precipitation can be controlled by a rational choice of the polymer structure such as the content of different monomers. A copolymer of *N*-hydroxymethyl acrylamide, *N*-isopropyl acrylamide (NIPAM) and butyl acrylate (BA) was modified with affinity ligand Cibacron Blue F3GA and used successfully for the affinity precipitation of lysozyme from egg white. A 28-fold purification with 80% lysozyme yield has been achieved using one simple unit operation (Shen and Cao, 2007). Among the three co-monomers, NIPAM provides the thermosensitive character to the copolymer, *N*-hydroxymethyl acrylamide brings the hydroxyl group to the polymer, which could be used for ligand coupling, while hydrophobic monomer BA helps to control the lower critical solution temperature (LCST) at an acceptable level of 28°C.

However the introduction of the hydrophilic and highly charged groups like IDA and NTA into the NIPAM molecular backbone changes the hydrophilicity/hydrophobicity balance of the polymer. The charged groups cause repulsive interaction and interfere with the hydrophobic interaction in poly-NIPAM (pNIPAM).This results in the shifting of the LCST to a higher temperature compared to that of pNIPAM homopolymer and gives a less efficient precipitation. A salt (e.g., sodium chloride) added during the precipitation screens the repulsive interaction between neighbouring charged groups on the polymer backbone and promotes hydrophobic interactions improving the precipitation. A less charged metal-binding ligand, imidazole

was successfully implemented in affinity precipitation as an alternative to IDA and NTA (Galaev *et al.*, 1997; Kumar *et al.*, 1998).

Individual imidazole groups randomly attached to the solid support are weak binding ligands in the chromatographic method. However, it was found as a part of the water soluble, flexible polymer that several imidazole ligands were capable of coming together to interact with the same metal ion. This provided sufficient strength for metal ion and protein binding (Galaev *et al.*, 1997). Introduction of a relatively hydrophilic imidazole comonomer influences the hydrophobic interactions in pNIPAM copolymer containing imidazole ligands and therefore requires higher temperatures for precipitation. The temperature of precipitation increases up to 60°C at the imidazole content of 24%. Loading the imidazole-NIPAM copolymer with metal ions, which induce a positive charge, makes the polymer more hydrophilic, thus completely preventing its precipitation. The ionic strength can be increased by adding a moderate amount of salt (about 0.4 M). This hinders the repulsive interactions and promotes the hydrophobic interactions leading to precipitation at mild conditions. When salt is added, the precipitation of Cu(II)-, Ni(II)-loaded copolymers of vinylimidazole (VI) and NIPAM are achieved at temperatures of between 20°C and 28°C which are lower than the LCST of pNIPAM (32°C). High salt concentration does not affect the formation of protein/metal ion/imidazole ligand complexes.

Separations with imidazole-containing copolymers have been used for the purification of proteins containing natural metal-ion binding sites and His-tag recombinant proteins, which bind to the metal immobilised onto the metal chelating group (Galaev *et al.*, 1997; Kumar *et al.*, 2003; Mattiasson *et al.*, 2007).

Besides the protein purification, a copper-containing copolymer of vinylimidazole with NIPAM (VI-NIPAM) has also been used for purification of plasmid DNA from RNA (Balan *et al.*, 2003). The single-stranded nucleic acid formed complexes with this metal through exposed purines and precipitated together with the polymer. It was found that in the absence of sodium chloride, DNA also interacted with the copolymer through the nucleic acid phosphate group interacting with metal. This non-specific interaction can be diminished by adding a moderate concentration of sodium chloride (up to 0.2 M). However, to maintain the polymer solubility in the presence of the salt, the separation mixture was kept in an ice bath. Polymer complexes with RNA were separated by precipitation with 0.8 M NaCl. The bound RNA was eluted with imidazole and the polymer was recovered by precipitation. The RNA separation strongly depended on the temperature, salt concentration and RNA structure. This opens the possibilities for finding the optimal separation conditions for a selective recovery of different RNA species. The binding strength of RNA to the VI-NIPAM polymer depends

on the RNA structure and molecular mass and thus could be controlled by changing the temperature and salt concentration.

PNIPAM with attached haptoglobin was synthesised for haemoglobin isolation from human blood (Stocker-Majd *et al.*, 2008). Attaching haptoglobin to pNIPAM does not change the interaction thermodynamics with haemoglobin. Compared to the affinity chromatography where the haptoglobin was attached to sepharose beads, the affinity precipitation provides better efficiency considering the number of the affinity ligands. It was found that more haemoglobin molecules bind to the same number of haptoglobin ligands when the latter is attached to soluble pNIPAM. Due to the pNIPAM flexibility, haptoglobin ligands are more accessible and there is less steric hindrance during the haemoglobin-haptoglobin interaction in the solution. Moreover, there is a possibility of binding more than one haemoglobin molecule to one haptoglobin ligand, which is provided by better accessibility of haptoglobin. Overall the affinity precipitation provides more efficient and cost-effective usage of expensive affinity ligands (Stocker-Majd *et al.*, 2008).

PNIPAM with an iminobiotin affinity tag was proposed for the affinity precipitation of avidin, streptavidin and avidin-carrying molecules (Garret-Flaudy and Freitag, 2001). The proposed technology provides better recovery of avidin compared with precipitation with polyligand, where about 31% of avidin activity was lost during the chromatography step. The avidin/biotin complex is very stable, which makes the product recovery difficult in conventional methods of separation. The recovery of the pNIPAM affinity ligand from avidin, however, was found to be straightforward due to the possibility of its thermoprecipitation (Garret-Flaudy and Freitag, 2001).

In addition to the commonly used synthetic polymers such as pNIPAM and poly(N-vinylcaprolactam) (PVCL), elastin-like polymers were introduced as thermoresponsive polymers for affinity precipitation (Shimazu *et al.*, 2003). An elastin-like polymer consists of the repeating penta-peptide, Val-Pro-Gly-Xaa-Gly (Xaa being any amino acid except proline). It undergoes reversible phase transition under temperature or salt concentration changes similar to pNIPAM (Shimazu *et al.*, 2003). An elastin-like polymer was used as a terminal tag for the facilitation of recombinant protein purification as attaching it to the hydrolase enzyme improves its purification. Purification, in excess of 1300-fold was achieved after two cycles of reversible changes of elastin-like polypeptide conformation precipitation induced by environmental changes (Shimazu *et al.*, 2003).

The elastin-like polypeptides have been also tested as carriers for metal-binding ligands (Stiborova *et al.*, 2003). The synthesised elastin-like polymer was chemically modified by introducing imidazole functionality. Purification of His-tag proteins, β-D-galactose and chloramphenicol acetyl transferase was performed in a manner similar to pNIPAM-vinyl imidazole systems.

Recently, SPs were used successfully for improving the refolding of recombinant proteins expressed in bacterial hosts, in the form of inactive and insoluble aggregates known as inclusion bodies (Gautam *et al.*, 2012). From the biotechnological perspective, the recovery of an active protein from inclusion bodies is achieved in two steps:

- the first step is solubilisation of the inclusion of the protein in the presence of denaturants;
- the second step is refolding upon decreasing concentration of the denaturant.

Polymers, including SPs, were used as refolding enhancers. The SPs work as pseudochaperonin and assist the protein folding down the correct refolding pathway. Apparently, SP interact with hydrophobic sites on the unfolded or partially folded protein molecule and prevent aggregation during refolding. A high throughput screening using 96-well plate format allowed identification of a suitable SP for obtaining a reasonable recovery of the active protein.

Another recently demonstrated separation application of SPs is flocculation. A thermosensitive polysaccharide was synthesised by graft-polymerisation of pNIPAM onto pullulan, a polysaccharide polymer consisting of maltotriose units, using Ce(IV) as the initiator. The thermosensitive polysaccharide induced flocculation of clay particles both below and above the lower critical temperature. This process, however, was more efficient with larger flocs forming above the LCST (Ghimici and Constantin, 2011).

13.3 Aqueous two-phase polymer systems formed by SPs for use in bioseparation

Aqueous two-phase systems have been used as a fast and effective process for separation of biomolecules (Gupta *et al.*, 1999). The two-phase polymer systems are commonly formed by using two incompatible polymer/polymer or polymer/salt systems. Poly(ethylene) glycol–dextran systems are commonly used in two-phase separation. Partitioning is a complex process and depends on the surface properties of the proteins. Depending on its hydrophilic/hydrophobic properties the target product concentrates in one of the phases, while the impurities remain in another phase and can be easily removed. The recovery of the protein from the phase-forming polymer is the main bottleneck of the purification process.

Two-phase aqueous systems with SPs were introduced as an alternative to poly(ethylene) glycol and dextran systems (Persson *et al.*, 2000). SPs could easily be recovered after separation owing to the reversible phase transition

controlled by small changes in the environment, such as pH or temperature. The possibility of easy recovery and SP reuse was the main reason for designing novel two-phase systems, as it reduces the cost of the whole purification process.

Random copolymers of ethylene oxide (EO) and propylene oxide (PO), collectively called EOPO polymers, are temperature-responsive polymers commonly used in two-phase systems. These polymers form a two-phase system in aqueous solution above the critical temperature which is known as a cloud point. Amino acids and peptides were separated using the EOPO two-phase system (Johansson *et al.*, 1998). At the temperature above the cloud point the copolymer formed a two-phase system comprising 40–60% polymer in water (polymer phase) and a nearly pure water phase (aqueous phase). Hydrophobic aromatic amino acids and peptides concentrated in the polymer phase while hydrophilic substances concentrated in the aqueous phase. The partitioning of the charged molecules was enhanced by adding salt (Johansson *et al.*, 1998). After the partitioning of biomolecules the polymer was easily recovered by heating the polymer phase above the critical temperature.

Apart from EO and PO, pNIPAM, PVCL and copolymers of *N*-isopropylacrylamide and *N*-vinyl caprolactam with vinyl imidazole were tested (Persson *et al.*, 2000). The copolymers with imidazole units change their properties with pH, becoming positively charged at lower pH levels and uncharged at levels above pH 7.0. This provides the possibility of directing the protein partitioning by changing the pH in the system rather than the more common addition of a salt (Persson *et al.*, 2000).

Hydrophobicity of EOPO copolymer was increased by modification with alkyl groups (C_{14}) (Persson *et al.*, 2000). It was found that the partitioning of bovine serum albumin (BSA) in the system of hydrophobically, modified EOPO and pNIPAM-vinylimidazole depending on the pH balance. The partition coefficients for BSA was 0.8 and 5.1 at pH 5.4 and pH 8, respectively. Thus BSA could be transferred from one polymer phase to another simply by changing the pH. This allows quantitative recovery of BSA (Persson *et al.*, 2000).

Introducing the metal-chelating ligand 1-vinylimidazole (VI) into a temperature-responsive *N*-vinylcaprolactam polymer (poly-VI-VCL) combines affinity interactions and two-phase systems. The affinity causes recombinant proteins to interact with imidazole groups loaded with Cu^{2+} forming the metal/protein complexes. This directed the partitioning of recombinant proteins into the poly-VI-VCL phase (Franco *et al.*, 1997; Pietruszka *et al.*, 2000). R-amylase inhibitors (Pietruszka *et al.*, 2000) and recombinant lactate dehydrogenase (LDH) (Franco *et al.*, 1997) were recovered using poly-VI-VCL with a yield of 75–80%. From polymer–

protein complexes the proteins were recovered by polymer precipitation in the presence of EDTA.

Separation of animal cells was achieved using two-phase systems containing pNIPAM conjugated with monoclonal antibodies (Kumar *et al.*, 2001). CD34-positive human acute myeloid leukaemia cells were specifically separated from human T lymphoma cells using poly-*N*-isopropylacrylamide conjugated with anti-CD34. About 80% of cells were partitioned in poly-*N*-isopropylacrylamide phase in the presence of NaCl below 0.1 M, and after temperature-induced precipitation about 75% cells recovery was achieved (Kumar *et al.*, 2001).

Recently light-sensitive polymers were applied in two-phase polymer systems (Li *et al.*, 2010). A light-sensitive polymer was synthesised by co-polymerisation of *N*-isopropylacrylamide (a temperature-responsive monomer) with *N*-vinyl-2-pyrrolidone and chlorophyllin sodium copper salt (light-sensitive monomers) (Wang *et al.*, 2008). The polymer was used in a two-phase polymer system for the separation of protein (BSA) and amino acid (L-Tyr). The conformational change of the copolymer was induced by light applying laser radiation at 488 nm. About 98% of the copolymer was recovered after the separation.

An interesting application of two-phase systems was developed for the bioconversion of cephalosporin-G (substrate) to 7-aminodesacetoxycephalosporanic acid (7-ADCA) (product), an important step in the synthesis of cephalosporin antibiotics(Li and Cao, 2011). This is an enzymatic reaction, which is inhibited by the excess of substrate as well as the product. The reaction slows down or stops at high concentration of cephalosporin-G and 7-ADCA, and so a system which will continuously introduce the substrate to the enzymatic solution and remove the product would be desirable. This challenge was solved by applying two-phase systems which provide partitioning of the enzyme, substrate and product. The partitioning of the substrate and the product between the two phases was adjusted by adding the $(NH_4)_2SO_4$. This allowed the concentration of substrate to be maintained at the desired maximum in the bottom phase, where the enzyme was. It also reduced the concentration of the product, which will be removed to the upper phase. The partitioning coefficient of the substrate and product between the two phases was adjusted to obtain the most efficient conversion. Partitioning of the enzyme to the bottom phase and most of the substrate and product to the upper phase, respectively, allows achievement of a 98.6% conversion ratio of cephalosporin-G at its initial concentration 12% (w/v)(Li and Cao, 2011). Temperature and pH-responsive polymers used for obtaining two-phase systems were easily recovered up to 97% by precipitation followed by centrifugation after changing pH and temperature.

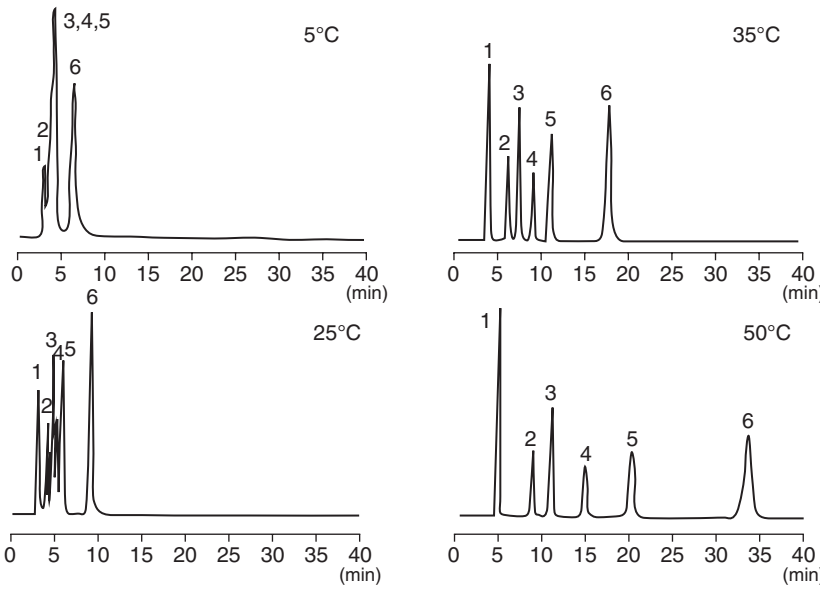
13.4 Chromatographic carriers with grafted SPs and adsorbents produced from SPs

The use of SPs in chromatography is based on the conformational changes of SPs grafted to the chromatographic matrices. The temperature driven transition from an expanded hydrophilic conformation to a compact hydrophobic one could be used for changing the size and volume of the pores available for the diffusion of the molecules to be separated. Alternatively, the changes in hydrophobicity of the grafted SP layer, below and above critical temperature, are exploited in so-called *temperature-modulated reversed-phase chromatography* (http://www.cellseed.com/product-e/005.html).

SP-modified chromatographic carriers were used in size exclusion chromatography (Gewehr *et al.*, 1992). Size exclusion chromatography uses a porous stationary phase to separate molecules according to their thermodynamic size. Small molecular mass substances (small thermodynamic size) penetrate into the porous stationary phase and have longer retention times, while bigger molecular mass substances cannot enter the porous material and are eluted earlier. Depending on the stationary phase pore size distribution, the separation of various molecular substances is achieved according to their molecular mass. Grafting pNIPAM to the surface of porous glass beads allows the pore size to be controlled by changing the temperature. The retention time shift is observed for dextrans when the temperature changes from 25 to 35°C. This shift depends on the initial pore size of the glass beads and molecular length of grafted pNIPAM. The authors suggested that the separation of a wide molecular range of dextrans could be achieved using this method (Gewehr *et al.*, 1992).

The temperature-responsive columns used for the separation of steroids were produced by grafting pNIPAM to the adsorbent (Kanazawa *et al.*, 1996). At temperatures above the transition point the pNIPAM-modified surface becomes hydrophobic. This provides better separation of the steroid molecules according to their log *P* values, where *P* is the partition coefficient of the substance in an octanol/water system. The separation of steroid molecules depends on the hydrophobicity of the stationary phase, which could be regulated by the temperature change. The temperature rise increases hydrophobicity of the modified stationary phase resulting in longer retention times and better separation of steroids according to their hydrophobicity. This is illustrated in Fig. 13.1, which presents elution profiles of five steroids: hydrocortisone, prednisolone, dexamethasone, hydrocortisone acetate and testosterone at different temperatures.

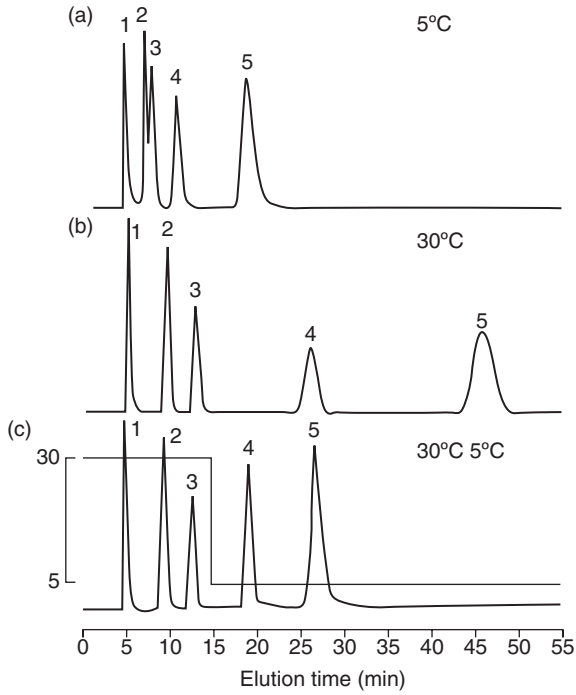
It was also demonstrated that the time for analysis could be shortened by applying a stepwise temperature gradient (Kikuchi and Okano, 2002). The separation of less hydrophobic steroids was carried out at the elevated



13.1 Chromatography of benzene and five steroids at different column temperature with water as mobile phase from pNIPAM-modified column. Peaks: 1 – benzene, 2 – hydrocortisone, 3 – prednisolone, 4 – dexamethasone, 5- hydrocortisone acetate; 6 – testosterone. Benzene was used as internal standard. (*Source*: Reprinted with permission from Kanazawa *, H., Yamamoto, K., Matsushima, Y., Takai, N., Kikuchi, A., Sakurai, Y. and Okano, T.* (1996). Temperature-responsive chromatography using poly(*N*-isopropylacrylamide)-modified silica. *Analytical Chemistry,* **68**, 100–105. Copyright (1996) American Chemical Society)) (Kanazawa *et al.*, 1996).

temperature of 30°C to achieve better resolution. The temperature was then lowered to 5°C which resulted in more hydrophobic steroids being efficiently eluted at shorter times (Fig. 13.2) (Kikuchi and Okano, 2002). Owing to the reversibility of conformational changes of the-temperature-responsive polymer, the hydrophilic/hydrophobic properties of the column could be changed within a few minutes, simply by varying the temperature. The whole separation was accomplished in aqueous solution without using organic solvents.

Chromatographic carriers with grafted temperature-responsive SPs have been introduced as an alternative to the following: reversed-phase chromatography, ion-exchange chromatography, hydrophobic interaction chromatography and combinations thereof, commonly used for the separation of large biomolecules as peptides and proteins (Kanazawa and Okano, 2011; Tan *et al.*, 2012). The separation of peptides and proteins using these techniques is based on the electrostatic and/or hydrophobic interaction of

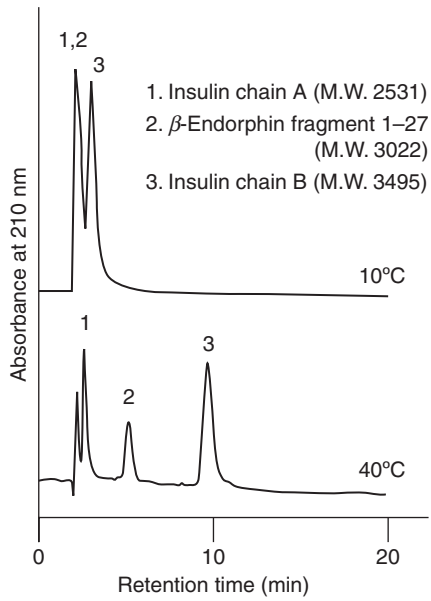


13.2 Effect of step-temperature gradient on steroid elution from pNIPAM-BMA (BMA 3.2 mol%)-modified column. Elution using water as mobile phase was done at 5°C (a), 30°C (b) and step-temperature gradient (c). (*Source*: Reproduced from Kikuchi and Okano (2002). Intelligent thermoresponsive polymeric stationary phases for aqueous chromatography of biological compounds. *Progress in Polymer Science,* **27**, 1165–1193. Copyright (2002), with permission from Elsevier.) (Kikuchi and Okano, 2002).

peptide groups with chromatographic carriers. Protein retention and elution are controlled by the composition of the mobile phase. In reversed-phase chromatography, organic solvents are added to the mobile phase to achieve protein separation. In both ion-exchange and hydrophobic interaction chromatography, electrostatic or hydrophobic interactions of proteins with chromatographic carriers are modulated by changing the buffer composition, pH or using a large amount of salt. However, the use of organic solvents or large amounts of salt has several disadvantages. Organic solvents used in reversed-phase chromatography are toxic and flammable thus limiting commercial applications of this technique. The large amounts of salt used in ion-exchange and, particularly, in hydrophobic interaction chromatography should be removed from the product, requiring additional purification and entailing additional costs associated with this step. Both organic solvent

usage and high salt concentrations may adversely affect the biological activity of proteins during the separation process. Modification of chromatographic carriers with a temperature-responsive polymer provides the means of controlling hydrophilic/hydrophobic properties of the carrier surface. This is achieved by a simple change of temperature rather than the mobile phase. This gives a possibility of performing the separation in aqueous solutions at mild conditions without the need of changing the mobile phase, making the whole process of separation more environmentally friendly and maintaining the biological activity of proteins. Changing the solute–adsorbent interactions by a simple procedure of controlling the temperature is an attractive feature for developing advanced bioseparation technologies that overcome the limitations of conventional separation processes.

The separation of three peptides: insulin chains A and B and β-endorphin fragments 1–27 was achieved under physiological conditions by a temperature change (Kanazawa and Okano, 2011). The temperature rise increases hydrophobicity of temperature-responsive carriers and hence the retention time of the peptides provides better separation (Fig. 13.3). As peptides have



13.3 Chromatography of mixture of the peptides: insulin chain A (1), insulin chain B (3) and β-endorphin fragments 1–27 (2) from pNIPAM-BMA-modified column (BMA 5%, eluent 0.9% NaCl aqueous solution). (*Source*: Reproduced from Kanazawa and Okano (2011). Temperature-responsive chromatography for the separation of biomolecules. *Journal of Chromatography A*, **1218**, 8738–8747. Copyright (2011), with permission from Elsevier.) (Kanazawa and Okano, 2011).

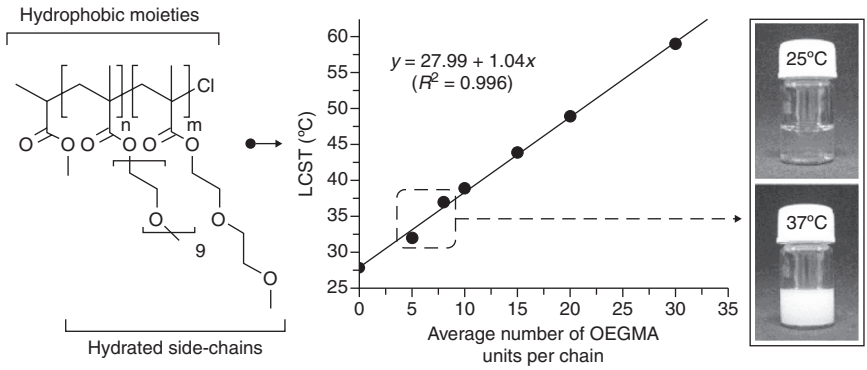
a different number of hydrophobic blocks, their retention time depends on their hydrophobicity and the strength of interaction with a stationary phase. At elevated temperatures the hydrophobic interactions of peptides with the stationary phase are stronger and the retention time is longer for more hydrophobic peptides. Thus by changing a single parameter (temperature) it was possible to control the interaction of peptides with the stationary phase and perform the separation without changing the mobile phase.

Chromatographic carriers with pNIPAM are the systems most frequently studied (Kikuchi and Okano, 2002; Kanazawa and Okano, 2011). pNIPAM was grafted to the silica carriers using both 'grafting to' and 'grafting from' techniques. The pre-formed pNIPAM is attached to the silica beads by standard amino-ester coupling ('grafting to'), or the NIPAM polymerisation is initiated from the silica surface modified with azo-initiator ('grafting from'). The 'grafting to' method provides better control of the molecular mass of the grafted polymer, while 'grafting from' yields a larger amount of the polymer grafted on the surface. Recently-developed technologies for controlled radical polymerisation, such as atom transfer radical polymerisation (ATRP) and reversible addition-fragmentation chain transfer (RAFT) polymerisation, allow preparation of the chromatographic substrate with well-defined grafted polymers using 'grafting from' techniques. Highly dense polymer brushes on the silica surface were obtained using these techniques. Controlled radical polymerisation provides the possibility of introducing a new block into a grafted polymer by re-initiating the polymerisation.

Other polymers as well as pNIPAM have been tested, such as structural isomers of pNIPAM, poly(2-oxazoline)s, PVCL and oligo(ethylene glycol) methacrylates (Miserez *et al.*, 2010; Tan *et al.*, 2009, 2012).

Special attention was given to the tuning of the polymer LCST by varying the polymer structure and composition. This was done by introducing additional hydrophobic blocks such as butyl methacrylate (BMA) or co-polymerisation of monomers with different LCST (Kikuchi and Okano, 2002; Lutz, 2008; Tan *et al.*, 2012).

Oligo(ethylene glycol)-based temperature-responsive polymers have attracted considerable attention recently as an alternative to pNIPAM (Lutz, 2008). These polymers have a carbon–carbon backbone with oligo(ethylene glycol) side-chains, which is different from a more common linear poly(ethylene oxide) structure (Fig. 13.4). Oligo(ethylene glycol) side-chain segments, however, account for a considerable part of the polymer and could make up to 85% of its mass. This makes the polymers water soluble and biocompatible. It was also shown that polymers with side-chain from 2 to 10 units have a lower LCST and combine the properties of both polyethylene glycol (PEG) and pNIPAM in a single macromolecule. Increasing EO length increases the hydrophilicity and therefore the LCST



13.4 Plots of the measured lower critical solution temperature (LCST) as a function of the theoretical average number of oligo(ethylene glycol) methacrylate (OEGMA)475 units per chain for a series of P(MEO2MA-co-OEGMA475) copolymers of various composition. (*Source*: Reproduced from Lutz (2008). Polymerization of oligo(ethylene glycol) (meth)acrylates: Toward new generations of smart biocompatible materials. *Journal of Polymer Science Part a-Polymer Chemistry*, **46**, 3459–3470. Copyright (2011), with permission from John Wiley and Sons.)

(Fig. 13.4); thus monomers with 2 and 3 EO units have a LCST of 26°C and 53°C, respectively (Lutz, 2008). Random copolymerisation of these two monomers allows the obtaining of copolymers with different LCST, which is dependent on the composition and structure of the copolymers (Lutz, 2008; Tan *et al.*, 2012).

Novel stationary phases with well-defined oligo(ethylene glycol)-grafted polymer were prepared by ATRP of (2-methoxyethoxy)ethyl methacrylate and oligo(ethylene glycol) methacrylate (Tan *et al.*, 2009). Temperature-responsive oligo(ethylene glycol)-based chromatography allows rapid and efficient separation of steroids and proteins in high pressure liquid chromatography (HPLC) mode (Tan *et al.*, 2009). Oligo(ethylene glycol)-grafted stationary phases as compared with the pNIPAM-grafted stationary phases provide better separation of hydrophilic compounds with close log *P* values (Tan *et al.*, 2012). This was explained by additional polar-type interactions of hydrophilic compounds with the oligo(ethylene glycol)-grafted stationary phase, which is absent in pNIPAM-grafted columns.

Chromatographic carriers with dual functionality responsive to pH and temperature were prepared by copolymerisation of a temperature-responsive monomer, with monomers bearing ionic groups (Kanazawa and Okano, 2011). Introduction of cationic or anionic groups allows the retention time of analytes to be controlled by manipulating electrostatic and hydrophobic interactions in the column. pH helps to control the electrostatic

interactions between the analyte and the column as well as influencing the hydrophobicity of the stationary phase as a result of protonation/deprotonation of the ion-exchange groups. For instance, protonation of cationic groups of *N*,*N*-dimethylaminopropylacrylamide (DMAPAAm) at lower pH increases the hydrophilicity of SP and raises LCST values, while deprotonation of cationic groups at higher pH increases the SP hydrophobicity resulting in a decreased LCST. On the other hand, at temperatures above LCST the ionic group is embedded inside the hydrophobic polymer and becomes less accessible to the electrostatic interactions. At temperatures below the LCST, ionic groups are easily accessible for electrostatic interactions due to the extended conformation of the polymer. Thus, electrostatic interactions could be easily controlled by changing the pH and temperature. This principle was used for the separation of oligonucleotides using a chromatographic column with pNIPAM-BMA-DMAPAAm copolymer (Nishio *et al.*, 2011). The chromatography was performed at pH 6.4 when the phosphorus-containing groups of oligonucleotides were deprotonated and in their anionic form provided electrostatic attraction with the cationic stationary phase. At temperatures above LCST the electrostatic interactions decreased, which resulted in the retention time of oligonucleotides decreasing.

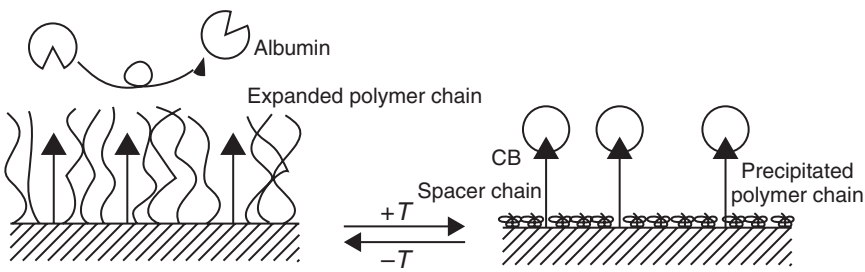
Temperature-responsive liquid chromatography-tandem mass spectrometry (LC-MS-MS) was used for the detection of drugs in human blood and urine (Kanno *et al.*, 2011). The separation and retention time of four cardiac glycosides were easily controlled by changing the temperature. It was possible to achieve the separation of four cardiac glycosides with the detection limit of 0.2–0.3 ng/L (Kanno *et al.*, 2011). Temperature-responsive chromatography gives the possibility for simultaneous detection of methyldigoxin and its main metabolite digoxin as well as compounds of different hydrophilicity/hydrophobicilty, in all the blood and urine samples.

In affinity chromatography, SPs were used to improve the elution and recovery of the target protein from the column. Affinity chromatography is often used for selective separation of proteins as target proteins are selectively adsorbed by the affinity ligand immobilised in the column. The recovery of the protein from the column requires changing pH and/or ionic strength to break down the affinity interaction between the protein and affinity ligand, which causes irreversible denaturation of the protein. Applying SP results in an easier recovery/separation under mild conditions. Temperature-responsive polymer PVCL was added to the eluent for the recovery of LDH from a Cibacron Blue dye-conjugated column (Galaev and Mattiasson, 1993, Galaev and Mattiasson, 1994) and it was found that 1% PVCL solution is more effective than 1.5 M KCl. Having amide polar groups, PVCL interacts strongly with Cibacron Blue displacing LDH. PVCL, as a temperature-responsive polymer, was recovered from protein solution

at an elevated temperature by centrifugation. It was demonstrated that the adsorption on the column could be regulated by the temperature. Firstly, the Cibacron Blue column was shielded with PVCL; then at temperatures below the LCST, PVCL was hydrated and it prevented all affinity sites from interaction with LDH. At elevated temperatures PVCL, shrank opening the affinity sites for the interaction. A crude extract containing LDH was applied at 40°C, above LCST of PVCL, the impurities were then eluted with 0.1 M KCl at 40°C, and LDH was eluted by lowering the temperature to 23°C with 90% recovery.

Another example is using the 'kicking-out' effect for protein separation. PNIPAM brushes were grafted to polymethacrylate beads together with an affinity ligand as shown in Fig. 13.5 (Kikuchi and Okano, 2002). The length of brushes and the affinity ligand spacer were designed in such a way that at lower temperatures, when the brushes are fully expanded, they shield the affinity ligand (Fig. 13.5). The adsorption of albumin onto the column was carried out at elevated temperatures when the grafted pNIPAM shrank exposing the affinity ligand for the interaction. The temperature was then lowered below the LCST and the expanded pNIPAM brushes 'kicked out' the adsorbed molecules of albumin.

Materials with grafted pNIPAM have been commercialised by a Japanese company CellSeed (http://www.cellseed.com). The company has two main product lines with thermosensitive polymers, the first of which is temperature-responsive cell plasticware, *UpCell*®. This allows cells that have been



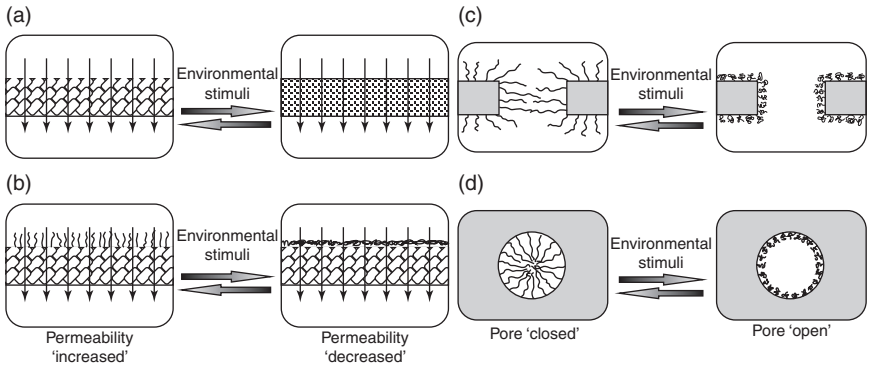
13.5 Selective adsorption/desorption control with temperature-responsive pNIPAM grafted together with affinity dye, Cibacron Blue. (*Source*: Reproduced from Kikuchi and Okano (2002). Intelligent thermoresponsive polymeric stationary phases for aqueous chromatography of biological compounds. *Progress in Polymer Science,* **27**, 1165–1193. Copyright (2002), with permission from Elsevier.) (Kikuchi and Okano, 2002).

grown to confluence to be detached from the plastic surface as a single sheet. These sheet-like clusters of cells are called 'cell sheets'. Using conventional techniques, cultured cell clusters were disassociated into their constituent cells when harvested using a proteolytic enzyme to detach them from the surface. The second product line is HPLC columns packed with aminopropyl silica grafted with pNIPAM (*Aqua Way Philic*) or cationic pNIPAM copolymer (*Aqua Way Cation*). *Aqua Way Philic* columns are designed for separation of proteins with different hydrophobicity in completely aqueous media. The elution of more hydrophobic proteins bound at an elevated temperature is achieved by the decrease of temperature, which reduces the hydrophobicity of the column. *Aqua Way Cation* columns were successfully used for the separation of proteins and anionic compounds such as adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP).

13.5 Membranes with SP-grafted pores

Stimuli-responsive membranes are widely used for controlled drug release and delivery, flux control and cell detachment; these applications are discussed in other chapters of this book. In this chapter we will focus mainly on the examples of separation of different substances using stimuli-responsive membranes rather than on the demonstration of a permeation on-off switch. One of the earliest reviews on using membranes in bioseparation was published in 1992 (Heath and Belfort, 1992). Recent works include examples where SPs contribute an additional function to the membrane (Chu *et al.*, 2011; Koros, 2004; Tokarev and Minko, 2010; Tokarev *et al.*, 2009).

Stimuli-responsive membranes could be prepared by various methods, which could be classified in two groups: membrane processing (solvent casting, phase inversion and radiation curing) and surface modification (grafting) (Wandera *et al.*, 2010). Membranes could be porous and non-porous and consist solely of SP or could have SP as one of the components (Fig. 13.6). SP could be embedded in the bulk of the membrane or grafted on the membrane surface or inside the pores. The substance permeation through the membrane depends on many factors but mainly on the membrane structure: thickness, density, porosity, hydrophilicity/hydrophobicity, etc. As the structure of non-stimuli-responsive membranes remains unchanged after the preparation, most commercially available membranes are single functioned; the separation is based on the size differences or diffusivity differences between the components of the mixture. A special group of membranes is used in membrane chromatography; they contain certain chemical groups, ligands, and are capable of specific interaction with target substances. The separation principles in this case are essentially the same as in chromatographic separations.

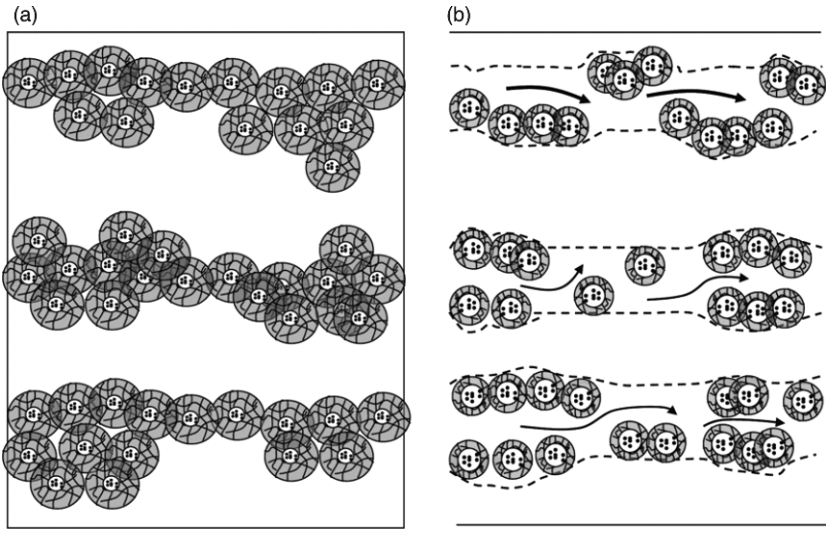


13.6 Membrane structures: stimuli-responsive hydrogel membrane (a), membrane with stimuli-responsive grafted brushes (b), stimuli-responsive membrane with porous substrate (c) and responsive gates (d). (*Source*: Reproduced from Chu, Xie and Ju (2011). Stimuli-responsive membranes: Smart tools for controllable mass-transfer and separation processes. *Chinese Journal of Chemical Engineering,* **19**, 891–903. Copyright (2012), with permission from Elsevier.) (Chu *et al.*, 2011).

Introduction of SP provides an additional tool for the variation of the membrane structure and thus changing/controlling the membrane permeability (Fig. 13.6). The possibility of altering the membrane permeability by varying external stimuli allows the opportunity for designing novel advanced materials (Chu *et al.*, 2011).

Recently, membranes with SP nanoparticles have been introduced which operate on a slightly different principle, as illustrated in Fig. 13.7. The membranes contain an ordered array of stimuli-responsive core-shell type magnetic polystyrene latex particles. The particles change their size in response to external stimuli, acting as 'on-off' switches or 'permeability valves', regulating the permeation through membrane channels.

Multi-component separation using stimuli-responsive membranes was introduced as an alternative to chromatographic separations (Huang *et al.*, 2009). The separation is based on the difference in solute size or the substance/membrane interaction (electrostatic, hydrophobic). Due to the short diffusion distance within membrane pores, the separation using membranes results in a lower pressure drop than in chromatographic columns. Moreover, membrane separations are less dependent on specific equipment and consume less buffer and energy, which makes them more cost efficient compared to chromatography (Koros, 2004). While the use of conventional membranes is limited to separation of compounds into two groups – permeable and non-permeable – the possibility of changing the SP membrane permeability during filtration allows multi-component separation to be achieved in one unit operation. A microporous polyvinylidene fluoride (PVDF) membrane



13.7 Schematic representation of the permeation mechanism through the channels of magnetic poly(styrene)-latex-pNIPAM smart nanocomposite membranes: (a) 'off' state below LCST and (b) 'on' state above LCST. (*Source*: Reproduced with permission from Csetneki, I., Filipcsei, G. and Zrinyi, M. (2006). Smart nanocomposite polymer membranes with on/off switching control. *Macromolecules,* **39**, 1939–1942. Copyright (2006) American Chemical Society.) (Csetneki *et al.*, 2006).

modified with poly-*N*-vinyl-lactams such as PVCL and poly(1-vinyl-pyrrolidone) cross-linked with bis-acrylamide has shown promising results for the separation of protein mixtures (Huang *et al.*, 2009). The phase transition of SP was induced by adding salt. Increase in the salt concentration results in the collapse of PVCL and opening of the pores. Permeation of BSA, human IgG, equine ferritin and thyroglobulin depended on the salt concentration. BSA (MW = 67 kDa) was removed first as it has high permeation, even though the pores were partially 'closed' by the expanded SP. Large proteins such as equine ferritin (MW = 440 kDa) and thyroglobulin (MW = 670 kDa) have low permeation through 'closed' pores; however, their permeation increased when pores opened at higher salt concentration. The authors also pointed out that other factors need to be considered when developing SP membranes for the separation. Most of the membranes have large polydispersity of pore sizes affecting the separation cut-off. Non-specific protein adsorption to the hydrophobic surface of the membrane, especially at high salt concentrations, needs to be considered as well as protein aggregation.

Chiral resolution of D,L-tryptophan was performed using the nylon-6 membrane with grafted pNIPAM containing β-cyclodextrin (β-CD) (Yang

et al., 2008). β-CD was used as the molecular recognition element capable of associating selectively with target molecules. L-Tryptophan was trapped by β-CD as it has a higher association constant than D-tryptophan, which instead permeates through the membrane. However, the dissociation of β-CD/target molecule complexes is not easy to achieve. It was demonstrated that, at temperatures above LCST, pNIPAM changes its conformation becoming hydrophobic and shrinks, which weakens the interaction of β-CD with L-tryptophan. Thus, the recovery of L-tryptophan and regeneration of the membrane could be more easily and efficiently achieved by changing the temperature. Similar work was done on adsorption/desorption of 8-anilino-1-naphthalene sulfonic acid ammonium salt using poly(*N*-isopropylacylamide-*co*-glycidylmethacrylate/cyclodextrin)-*grafted*-polyethyleneterephthalate membranes (Xie *et al.*, 2009).

An interesting example of exploiting pNIPAM-grafted-polypropylene for cell separation was presented by Okamura *et al.*, (2005). PNIPAM-grafted polypropylene membrane was modified by physical adsorption of the monoclonal antibody against mouse CD80 cells at temperatures above LCST 37°C when grafted pNIPAM had collapsed hydrophobic conformation. The CD80 cells attached specifically to the membrane due to the interaction with adsorbed antibodies and were released from the membrane by lowering the temperature to 4°C. About 70% enrichment of specific cells was achieved.

pH-sensitive SPs undergo conformational changes when pH is altered. For example, (co)polymers of (meth)acrylic acid have an expanded conformation as polyanions at alkaline pH and compacted conformation in a neutral form at acidic pH. (Co)polymers of 2-dimethylaminoethyl methacrylate or vinyl pyridine are expanded as polycations at acidic pH and compact in a neutral form at alkaline pH. Membranes composed of blends of pH-sensitive SPs, or having SPs grafted in the pores, change their permeability with pH. The on-off type switch of permeation has been demonstrated for different systems and, more recently, has been extensively reviewed (Zhao *et al.*, 2011). So far the examples of actual separations using pH-sensitive membranes are very few. On the other hand, one should distinguish membranes with pH-sensitive SPs from membranes having ionic groups, which are used widely in the food and pharmaceutical industries in an ion-exchange mode. Strong anion exchange membranes (Mustang Q) have been reported to remove residual plasmid DNA, negatively-charged proteins and viral particles from the pharmacological protein preparations. (http://www.pall.com/main/Biopharmaceuticals/Product.page?id=33053).

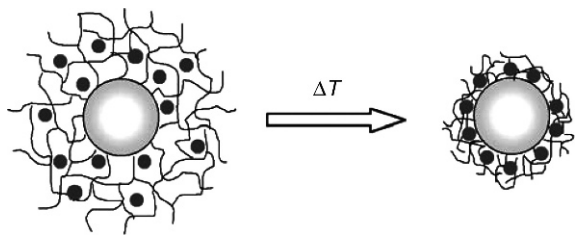
Apart from membranes altering their permeability in response to temperature change or a combination of salt concentration and temperature change, other membranes have been developed whose permeability is influenced by electric field, magnetic field or illumination with light of particular

wavelength. The production and properties of these membranes have been extensively reviewed recently in Wandera *et al.* (2010). However, in most cases only an on-off switch of solute permeation was demonstrated rather than separation of different solutes based on their properties. One such example of actual separation is presented in the work of Kokufuta *et al.* (1995) on electrically controlled separation of maleic acid and fumaric acid using polyelectrolyte membranes. They are prepared by iterative freezing–thawing of an aqueous solution containing 10% (w/w) each of polyacrylic acid and polyvinylalcohol.

13.6 Use of smart polymers in catalysis

Transformation of SPs, swelling–shrinking, was used to control the activity of the embedded catalysts (Lu *et al.*, 2006, 2009a). Metal nanoparticles such as Ag, Au, Rh or Pt were embedded inside the pNIPAM shell and their exposure to the medium meant that their catalytic activity was dependent on the temperature-induced conformational changes of the polymer (Fig. 13.8).

Metal nanoparticles embedded inside temperature-responsive polymer brushes (Lu *et al.*, 2006) or cross-linked pNIPAM shell (Lu *et al.*, 2009a) were tested for the oxidation of alcohols to corresponding aldehydes or ketones (Lu *et al.*, 2009a). The nanoparticles were fully accessible to the reactants at the temperature below the LCST of pNIPAM. Swelling–shrinking could be

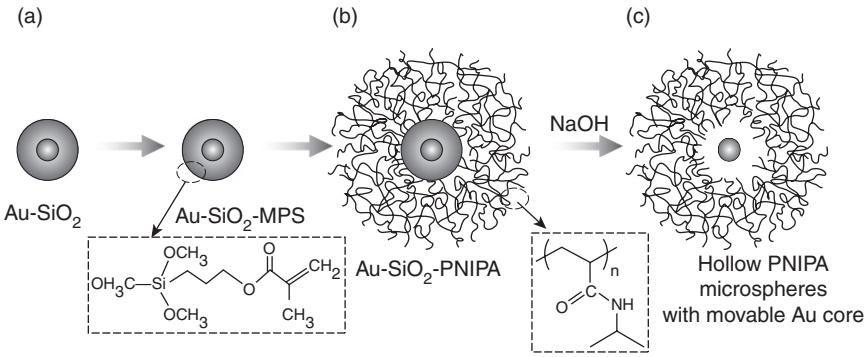


13.8 Poly(styrene) composite particles consisting of thermosensitive core-shell of pNIPAM in which Ag nanoparticles are embedded. The composite particles are suspended in water which swells the thermosensitive network attached to the surface of core particles. In this state the reagents can diffuse freely to the nanoparticles that act as catalysts. At higher temperature ($T > 30$°C) the network shrinks and the catalytic activity of the nanoparticles is strongly diminished. (*Source*: Reproduced from Lu, Mei, Drechsler and Ballauff (2006). Thermosensitive core-shell particles as carriers for Ag nanoparticles: Modulating the catalytic activity by a phase transition in networks. *Angewandte Chemie-International Edition,* **45**, 813–816. Copyright (2005), with permission from John Wiley and Sons.) (Lu *et al.*, 2006).

repeated many times without affecting their catalytic activity. Embedding the nanoparticles inside the SP shell also prevents their agglomeration.

Similar particulate systems with a polyelectrolyte polymer core have been developed for the immobilisation of nanoparticles and enzymes (Lu *et al.*, 2009b). In both cases the core of the particles was used for stabilising the catalytic systems, preventing their agglomeration and for easier handling and recovery after the completion of the reaction. In the case of the enzymes, polyelectrolyte polymer core particles present a better way of enzyme immobilisation than immobilisation on solid surfaces. The mild conditions inside the polyelectrolyte core preserve the native conformation and biological activity of enzymes.

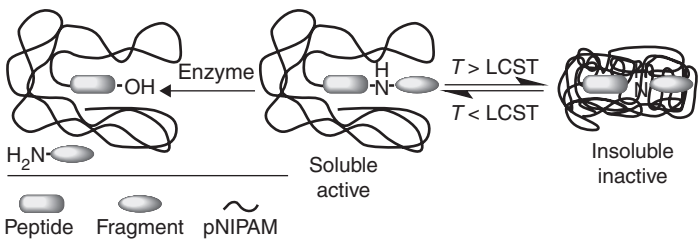
A single Au nanoparticle inside the pNIPAM shell catalytic system, a so-called hybrid yolk–shell nanostructure, was synthesised as shown in Fig. 13.9 (Wu *et al.*, 2012). This system showed catalytic activity in the reduction of 4-nitrophenol and nitrobenzene with $NaBH_4$, in which the selectivity of hydrogenation depended on temperature. The reduction of 4-nitrophenol was much faster at lower temperature, whereas nitrobenzene reacted faster at higher temperature. Both compounds are of similar size; thus the changes in pNIPAM core conformation from a swollen to a shrunken state could not affect the diffusion of the reactants through the pNIPAM core. However, 4-nitrophenol is more hydrophilic than nitrobenzene. The interaction of the reactants with pNIPAM in its hydrophilic and hydrophobic state could be a reason for the temperature dependence of the selectivity of catalysis.



13.9 Illustration of the generation of Au-pNIPAM yolk–shell composite particles: (a) modification of the Au-SiO_2 core–shell particles with 3-(trimethoxysilyl)propyl methacrytate (MPS) (b) formation of the pNIPAM shell on the Au-SiO_2 particle surface (c) removing the silica of the Au-SiO_2-pNIPAM composites by etching in a highly concentrated NaOH solution. (*Source*: Reproduced with permission from Wu, Dzubeiella, Kaiser, Drechsler, Guo, Ballauff and Lu (2012). Thermosensitive Au-PNIPA Yolk–Shell Nanoparticles with Tunable Selectivity for Catalysis. *Angewandte Chemie International Edition,* **51**, 2229–2233. Copyright (2012) John Wiley and Sons.) (Wu *et al.*, 2012).

Catalytic properties of poly(*N*-vinylcaprolactam-*co*-1-vinylimidazole) and poly(*N*-isopropylacrylamide-*co*-1-vinylimidazole) in the hydrolysis of *p*-nitrophenyl acetate were studied by Okhapkin *et al.* (2004). The polymers contain imidazole groups and behave similarly to enzymes of the hydrolase type. The catalytic reaction rate was found to be higher than expected based on a prediction by Arrhenius-type behaviour. This type of behaviour was observed at the temperature above LCST of the copolymer, when it formed aggregates. This was explained by increasing adsorption of the substrate onto the copolymer aggregates at a higher temperature as a result of increased affinity of *p*-nitrophenyl acetate to the copolymer.

Another opportunity, which SPs provide in catalysis, is to combine the advantages of homogeneous catalysis (no diffusion limitations and no need for stirring) with those of heterogeneous catalysis (easy separation of the catalyst from the reaction media when the conversion is completed). This allows manifold catalyst reuse. Cellulase was covalently immobilised on a pH-sensitive copolymer of methacrylic acid and methylmethacrylate, Eudragit® L-100 (Zhang *et al.*, 2010). Two modelling approaches – response surface methodology and artificial neural network – were applied to investigate the effects of pH, the coupling agent (carbodiimide) concentration and the coupling time of the activity yield of immobilised cellulase. Results showed that simulation and prediction accuracy of artificial neural network modelling was higher compared to that of the response surface methodology. High performance of the cellulase immobilised onto Eudragit for repeated hydrolysis of insoluble cellulose was achieved due to easy recovery/reuse of the enzyme. The productivity remained above 50% of the initial value after reusing immobilised cellulase in the other five cycles. Some loss in productivity was explained by the detachment of the enzyme molecules from Eudragit due to weak binding, unavoidable enzyme deactivation



13.10 Reversible switching of the activity in pNIPAM–peptide conjugates. (*Source*: Reproduced from Molawi and Studer (2007). Reversible switching of substrate activity of pNIPAM–peptide conjugates. *Chemical Communications*, 5173–5175. Copyright (2007), with permission from Royal Society of Chemistry.) (Molawi and Studer, 2007).

during reaction and separation process, and enzyme loss due to incomplete desorption of the enzyme from the separated substrate.

A temperature-induced phase switch of the smart pNIPAM tail of these novel bioconjugates was demonstrated to alter the substrate activity towards enzymatic hydrolysis of the pNIPAM–peptide conjugates (Fig. 13.10) (Molawi and Studer, 2007).

In conclusion, combination of biocatalysts with SPs provides novel opportunities for controlling (bio)catalytic reactions. However, there is still a long way to go before these systems could mature to industrial applications.

13.7 Conclusion and future trends

As indicated recently in the highlight paper in the *Journal of Polymer Science Part B: Polymer Physics* (Kirsebom *et al.*, 2011), the tendency in the smart polymer area is to produce new materials with more elaborate and better controlled architecture which are more sensitive and respond even faster and in a more immediate (all-or-nothing) way. Special attention is given to the development of robust systems capable of long-term performance without losing their sensitivity or efficiency. This approach has led to the maturing of some purification systems with SPs to commercial applications. An example is a range of temperature-modulated, reversed-phase HPLC columns *Aqua Way* produced by CellSeed Inc. (Tokyo, Japan) (http://www.cellseed.com/product-e/005.html). However, it should be pointed out that many methods presented in the chapter are designed and tested on a lab scale only. The challenge will be the translation of these methods to commercially successful, large-scale applications. Another direction is the development of multi-stimuli-responsive complex materials and materials mimicking their nature, such as designing the membranes with selective and controllable permeation similar to cell membranes. One can envisage more and more SP systems designed and introduced into every day research and ultimately into commercial production.

13.8 References

Balan, S., Murphy, J., Galaev, I., Kumar, A., Fox, G. E., Mattiasson, B. and Willson, R. C. (2003). Metal chelate affinity precipitation of RNA and purification of plasmid DNA. *Biotechnology Letters,* **25**, 1111–1116.

Block, H., Maertens, B., Spriestersbach, A., Brinker, N., Kubicek, J., Fabis, R., Labahn, J. and Schafer, F. (2009). Immobilized-metal affinity chromatography (IMAC): A review. *Methods in Enzymology,* **463**, 439–473.

Chu, L. Y., Xie, R. and Ju, X. J. (2011). Stimuli-responsive membranes: Smart tools for controllable mass-transfer and separation processes. *Chinese Journal of Chemical Engineering,* **19**, 891–903.

Franco, T. T., Galaev, I. Y., Hattikaul, R., Holmberg, N., Bulow, L. and Mattiasson, B. (1997). Aqueous two-phase system formed by thermoreactive vinyl imidazole vinyl caprolactam copolymer and dextran for partitioning of a protein with a polyhistidine tail. *Biotechnology Techniques,* **11**, 231–235.

Galaev, I. Y. and Mattiasson, B. (1993). Effect of synthetic-polymers, poly(N-vinyl pyrrolidone) and poly(N-vinyl caprolactam), on elution of lactate-dehydrogenase bound to blue Sepharose. *Journal of Chromatography,* **648**, 367–371.

Galaev, I. Y. and Mattiasson, B. (1994). Poly(N-vinylpyrrolidone) shielding of matrices for dye-affinity chromatography – improved elution of lactate dehydrogenase from Sepharose and secondary alcohol dehydrogenase from scarlet Sepharose. *Journal of Chromatography A,* **662**, 27–35.

Galaev, I. Y. and Mattiasson, B. (eds.) (2002). *Smart Polymers for Bioseparation and Bioprocessing,* London: Taylor & Francis.

Galaev, I. Y. and Mattiasson, B. (eds.) (2007). *Smart Polymers. Applications in Biotechnology and Biomedicine.,* Boca Raton, USA: CRC Press, Taylor & Francis Group.

Galaev, I. Y., Kumar, A., Agarwal, R., Gupta, M. N. and Mattiasson, B. (1997). Imidazole – A new ligand for metal affinity precipitation – Precipitation of kunitz soybean trypsin inhibitor using Cu(II)-loaded copolymers of 1-vinylimidazole with N-vinylcaprolactam or N-isopropylacrylamide. *Applied Biochemistry and Biotechnology,* **68**, 121–133.

Garret-Flaudy, F. and Freitag, R. (2001). Use of the avidin (imino)biotin system as a general approach to affinity precipitation. *Biotechnology and Bioengineering,* **71**, 223–234.

Gautam, S., Dubey, P., Singh, P., Kesavardhana, S., Varadarajan, R. and Gupta, M. N. (2012). Smart polymer mediated purification and recovery of active proteins from inclusion bodies. *Journal of Chromatography A,* **1235**, 10–25.

Gewehr, M., Nakamura, K., Ise, N. and Kitano, H. (1992). Gel-permeation chromatography using porous-glass beads modified with temperature-responsive polymers. *Makromolekulare Chemie-Macromolecular Chemistry and Physics,* **193**, 249–256.

Ghimici, L. and Constantin, M. (2011). Novel thermosensitive flocculanting agent based on pullulan. *Journal of Hazardous Materials,* **192**, 1009–1016.

Gupta, M. N. and Mattiasson, B. (1994). Affinity precipitation. *In:* Blackie Academic and Professional (ed.) *Highly Selective Separations in Biotechnology.* London: G. Street.

Gupta, R., Bradoo, S. and Saxena, R. K. (1999). Aqueous two-phase systems: An attractive technology for downstream processing of biomolecules. *Current Science,* **77**, 520–523.

Heath, C. and Belfort, G. (1992). Synthetic membranes in biotechnology: Realities and possibilities. *In:* Tsao, G. (ed.) *Bioseparation.* Berlin/Heidelberg: Springer.

Huang, R., Kostanski, L. K., Filipe, C. D. M. and Ghosh, R. (2009). Environment-responsive hydrogel-based ultrafiltration membranes for protein bioseparation. *Journal of Membrane Science,* **336**, 42–49.

Johansson, H. O., Karlstrom, G. and Tjerneld, F. (1998). Separation of amino acids and peptides by temperature induced phase partitioning. Theoretical model for partitioning and experimental data. *Bioseparation,* **7**, 259–267.

Kanazawa, H. and Okano, T. (2011). Temperature-responsive chromatography for the separation of biomolecules. *Journal of Chromatography A,* **1218**, 8738–8747.

Kanazawa, H., Yamamoto, K., Matsushima, Y., Takai, N., Kikuchi, A., Sakurai, Y. and Okano, T. (1996). Temperature-responsive chromatography using poly(N-isopropylacrylamide)-modified silica. *Analytical Chemistry,* **68**, 100–105.

Kanno, S., Watanabe, K., Yamagishi, I., Hirano, S., Minakata, K., Gonmori, K. and Suzuki, O. (2011). Simultaneous analysis of cardiac glycosides in blood and urine by thermoresponsive LC-MS-MS. *Analytical and Bioanalytical Chemistry,* **399**, 1141–1149.

Kikuchi, A. and Okano, T. (2002). Intelligent thermoresponsive polymeric stationary phases for aqueous chromatography of biological compounds. *Progress in Polymer Science,* **27**, 1165–1193.

Kirsebom, H., Galaev, I. Y. and Mattiasson, B. (2011). Stimuli-responsive polymers in the 21st century: Elaborated architecture to achieve high sensitivity, fast response, and robust behavior. *Journal of Polymer Science Part B-Polymer Physics,* **49**, 173–178.

Kokufuta, E., Yamauchi, T. and Osada, Y. (1995). Electrically controlled separation of maleic acid and fumaric acid through a poly(vinyl alcohol) poly(acrylic acid) composite membrane. *Polymer Gels and Networks,* **3**, 397–406.

Koros, W. J. (2004). Evolving beyond the thermal age of separation processes: Membranes can lead the way. *AIChE Journal,* **50**, 2326–2334.

Kumar, A., Galaev, I. Y. and Mattiasson, B. (1998). Affinity precipitation of α-amylase inhibitor from wheat meal by metal chelate affinity binding using cu(II)-loaded copolymers of 1-vinylimidazole with N-isopropylacrylamide. *Biotechnology and Bioengineering,* **59**, 695–704.

Kumar, A., Kamihira, M., Galaev, I. Y., Mattiasson, B. and Iijima, S. (2001). Type-specific separation of animal cells in aqueous two-phase systems using antibody conjugates with temperature-sensitive polymers. *Biotechnology and Bioengineering,* **75**, 570–580.

Kumar, A., Mattiasson, B. and Galaev, I. Y. (2007). Smart polymers in affinity precipitation of proteins. *In:* Galaev, I. Y. and Mattiasson, B. (eds.) *Smart Polymers. Applications in Biotechnology and Biomedicine.* CRC Press.

Kumar, A., Wahlund, P. O., Kepka, C., Galaev, I. Y. and Mattiasson, B. (2003). Purification of histidine-tagged single-chain Fv-antibody fragments by metal chelate affinity precipitation using thermoresponsive copolymers. *Biotechnology and Bioengineering,* **84**, 494–503.

Li, H. and Cao, X. J. (2011). Bioconversion of cephalosporin-G to 7-ADCA in a pH-thermo sensitive recycling aqueous two-phase systems. *Process Biochemistry,* **46**, 1753–1758.

Li, X., Wan, J. F. and Cao, X. J. (2010). Preliminary application of light-pH sensitive recycling aqueous two-phase systems to purification of lipase. *Process Biochemistry,* **45**, 598–601.

Lu, Y., Mei, Y., Drechsler, M. and Ballauff, M. (2006). Thermosensitive core-shell particles as carriers for Ag nanoparticles: Modulating the catalytic activity by a phase transition in networks. *Angewandte Chemie-International Edition,* **45**, 813–816.

Lu, Y., Proch, S., Schrinner, M., Drechsler, M., Kempe, R. and Ballauff, M. (2009a). Thermosensitive core-shell microgel as a 'nanoreactor' for catalytic active metal nanoparticles. *Journal of Materials Chemistry,* **19**, 3955–3961.

Lu, Y., Wittemann, A. and Ballauff, M. (2009b). Supramolecular structures generated by spherical polyelectrolyte brushes and their application in catalysis. *Macromolecular Rapid Communications,* **30**, 806–815.

Lutz, J. F. (2008). Polymerization of oligo(ethylene glycol) (meth)acrylates: Toward new generations of smart biocompatible materials. *Journal of Polymer Science Part a-Polymer Chemistry,* **46**, 3459–3470.

Mattiasson, B., Kumar, A., Ivanov, A. E. and Galaev, I. Y. (2007). Metal-chelate affinity precipitation of proteins using responsive polymers. *Nature Protocols,* **2**, 213–220.

Miserez, B., Lynen, F., Wright, A., Euerby, M. and Sandra, P. (2010). Thermoresponsive poly(N-vinylcaprolactam) as stationary phase for aqueous and green liquid chromatography. *Chromatographia,* **71**, 1–6.

Molawi, K. and Studer, A. (2007). Reversible switching of substrate activity of poly-N-isopropylacrylamide peptide conjugates. *Chemical Communications*, 5173–5175.

Nishio, T., Ayano, E., Suzuki, Y., Kanazawa, H. and Okano, T. (2011). Separation of phosphorylated peptides utilizing dual pH- and temperature-responsive chromatography. *Journal of Chromatography A,* **1218**, 2079–2084.

Okamura, A., Itayagoshi, M., Hagiwara, T., Yamaguchi, M., Kanamori, T., Shinbo, T. and Wang, P. C. (2005). Poly(N-isopropylacrylamide)-graft-polypropylene membranes containing adsorbed antibody for cell separation. *Biomaterials,* **26**, 1287–1292.

Okhapkin, I. M., Bronstein, L. M., Makhaeva, E. E., Matveeva, V. G., Sulman, E. M., Sulman, M. G. and Khokhlov, A. R. (2004). Thermosensitive imidazole-containing polymers as catalysts in hydrolytic decomposition of p-nitrophenyl acetate. *Macromolecules,* **37**, 7879–7883.

Persson, J., Johansson, H.-O., Galaev, I., Mattiasson, B. and Tjerneld, F. (2000). Aqueous polymer two-phase systems formed by new thermoseparating polymers. *Bioseparation,* **9**, 105–116.

Pietruszka, N., Galaev, I. Y., Kumar, A., Brzozowski, Z. K. and Mattiasson, B. (2000). New polymers forming aqueous two-phase polymer systems. *Biotechnology Progress,* **16**, 408–415.

Senstad, C. and Mattiasson, B. (1989). Affinity-precipitation using chitosan as ligand carrier. *Biotechnology and Bioengineering,* **33**, 216–220.

Shen, L. L. and Cao, X. J. (2007). Synthesis of thermo-sensitive polyacrylarnide derivatives for affinity precipitation and its application in purification of lysozyme. *Biochemical Engineering Journal,* **33**, 66–71.

Shimazu, M., Mulchandani, A. and Chen, W. (2003). Thermally triggered purification and immobilization of elastin-OPH fusions. *Biotechnology and Bioengineering,* **81**, 74–79.

Stiborova, H., Kostal, J., Mulchandani, A. and Chen, W. (2003). One-step metal-affinity purification of histidine-tagged proteins by temperature-triggered precipitation. *Biotechnology and Bioengineering,* **82**, 605–611.

Stocker-Majd, G., Hilbrig, F. and Freitag, R. (2008). Extraction of haemoglobin from human blood by affinity precipitation using a haptoglobin-based stimuli-responsive affinity macroligand. *Journal of Chromatography A,* **1194**, 57–65.

Tan, I., Roohi, F. and Titirici, M. M. (2012). Thermoresponsive polymers in liquid chromatography. *Analytical Methods,* **4**, 34–43.

Tan, I., Zarafshani, Z., Lutz, J. F. and Titirici, M. M. (2009). PEGylated chromatography: Efficient bioseparation on silica monoliths grafted with smart biocompatible polymers. *Acs Applied Materials and Interfaces,* **1**, 1869–1872.

Tokarev, I. and Minko, S. (2010). Stimuli-responsive porous hydrogels at interfaces for molecular filtration, separation, controlled release, and gating in capsules and membranes. *Advanced Materials,* **22**, 3446–3462.

Tokarev, I., Motornov, M. and Minko, S. (2009). Molecular-engineered stimuli-responsive thin polymer film: a platform for the development of integrated multifunctional intelligent materials. *Journal of Materials Chemistry,* **19**, 6932–6948.

Wandera, D., Wickramasinghe, S. R. and Husson, S. M. (2010). Stimuli-responsive membranes. *Journal of Membrane Science,* **357**, 6–35.

Wang, W., Wan, J. F., Ning, B., Xia, J. N. and Cao, X. J. (2008). Preparation of a novel light-sensitive copolymer and its application in recycling aqueous two-phase systems. *Journal of Chromatography A,* **1205**, 171–176.

Wu, S., Dzubiella, J., Kaiser, J., Drechsler, M., Guo, X., Ballauff, M. and Lu, Y. (2012). Thermosensitive Au-PNIPA yolk–shell nanoparticles with tunable selectivity for catalysis. *Angewandte Chemie International Edition,* **51**, 2229–2233.

Xie, R., Zhang, S. B., Wang, H. D., Yang, M., Li, P. F., Zhu, X. L. and Chu, L. Y. (2009). Temperature-dependent molecular-recognizable membranes based on poly(N-isopropylacrylamide) and beta-cyclodextrin. *Journal of Membrane Science,* **326**, 618–626.

Yang, M., Chu, L. Y., Wang, H. D., Xie, R., Song, H. and Niu, C. H. (2008). A thermoresponsive membrane for chiral resolution. *Advanced Functional Materials,* **18**, 652–663.

Zhang, Y., Xu, J. L., Yuan, Z. H., Xu, H. J. and Yu, Q. (2010). Artificial neural network-genetic algorithm based optimization for the immobilization of cellulase on the smart polymer Eudragit L-100. *Bioresource Technology,* **101**, 3153–3158.

Zhao, C. S., Nie, S. Q., Tang, M. and Sun, S. D. (2011). Polymeric pH-sensitive membranes-A review. *Progress in Polymer Science,* **36**, 1499–1520.

14

Smart polymers for textile applications

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Abstract: Smart polymers are materials which can show noticeable changes in their properties with environmental stimulation. Novel functionalities can be delivered to textiles by integrating smart polymers into them. Smart polymers including thermal-, moisture- and light-responsive polymers, and thermal- and pH-responsive hydrogels, have been applied to textiles to improve or achieve textile smart functionalities. The functionalities include aesthetic appeal, comfort, drug release, fantasy design (colour changing), wound monitoring, smart wetting properties, and protection against extreme environmental variations. In this chapter, the applications of smart polymers in textiles and the clothing sector are elucidated; the associated constraints on fabrication of textiles and their potential applications in the near future are discussed.

Key words: thermal-responsive polymer, moisture-responsive polymer, thermal-responsive hydrogel, pH-responsive hydrogel, smart textiles.

14.1 Introduction

Smart materials can sense the environment and/or their own state, make a judgment and then respond by changing their functions according to a predetermined purpose.[1] Such smart functions are distinct from smart systems because they do not rely on the complicated sense-response structure of a feedback system. Instead, smart materials are intrinsically sensitive to changes in their environment such as temperature, optical wavelength, absorbed gas molecules or pH values.

Smart polymers such as shape memory polymers (SMPs), phase change materials (PCMs), colour change polymers and intelligent polymer hydrogels, have developed rapidly in the past few decades. Because they are easy to process, and are light and flexible, smart polymers have been an important material in textile processing. SMPs have the capability to memorize a permanent shape and to be programmed to assume one or many temporary shapes; upon exposure to an external stimulus, they spontaneously recover their original permanent shape. For example, a closed flower (temporary shape) made of a SMP is fixed at a lower temperature and recovers from

the closed state to an open flower (original shape) when the temperature is increased above its switch temperature.

PCMs have the ability to absorb and emit heat energy without changing temperature themselves. These waxes include eicosane, octadecane, nonadecane, heptadecane and hexadecane, which all have different freezing and melting points and, when combined in a microcapsule, will store heat energy and emit heat energy and maintain their temperature range of 30~34°C, which is comfortable for the body.

Smart polymer hydrogels undergo reversible volume change responding to a small variation in solution conditions (external stimuli), such as temperature,[2–7] pH[2,8,9] and solvent compositions.[10] Many hydrogels such as poly(N-substituted acrylamide), poly(N-vinyl alkylamide), poly(vinyl methyl ether), and poly(ethylene glycol-co-propylene glycol) have been studied and utilized for diverse textile applications. Poly(N-isopropyl acrylamide) (PNIPAAm) is an intensively concentrated temperature-sensitive polymer which has a simultaneously hydrophilic and hydrophobic structure. Because of its sharp temperature-induced transition, PNIPAAm (and in particular the PNIPAAm hydrogel) has been developed into stimuli-sensitive textiles.

Polymers which change their visible optical properties in response to external stimuli have aroused the growing interest of researchers. According to the external stimulus, these polymers are classified as:

- thermochromic (stimulus: temperature);
- photochromic (stimulus: light);
- electrochromic (stimulus: electric field);
- piezochromic (stimulus: pressure);
- ionochromic (stimulus: ion concentration); and
- biochromic (stimulus: biochemical reaction).

Most smart polymers can be triggered in a variety of ways such as the response of SMPs to thermal, chemical, magnetic and water stimuli. Another example is the way a hydrogel can respond to pH, heat, light, magnetic fields, etc. These diverse stimuli make it possible to use smart polymers in different applications. Smart polymers used in textiles usually appear in various forms such as film, fibre, solution or gel to meet different processing requirements in textiles.

14.2 Types of smart polymers for textile applications

Different types of smart polymers have significant effects on the applications used in textile technology. There are a variety of smart polymers that can be used with specific processing techniques, such as finishing, spinning, weaving or laminating. It is important to use the correct type of smart polymer for

specific textile applications. There are five common forms or shapes used to describe the smart polymers mentioned in the introduction. The five forms are:

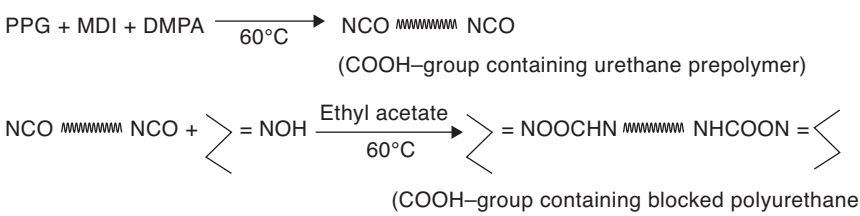
- solution
- microcapsules
- gel
- film/foam
- fibre
- nonwoven.

These forms are either in direct or intermediate states when incorporated into textiles.

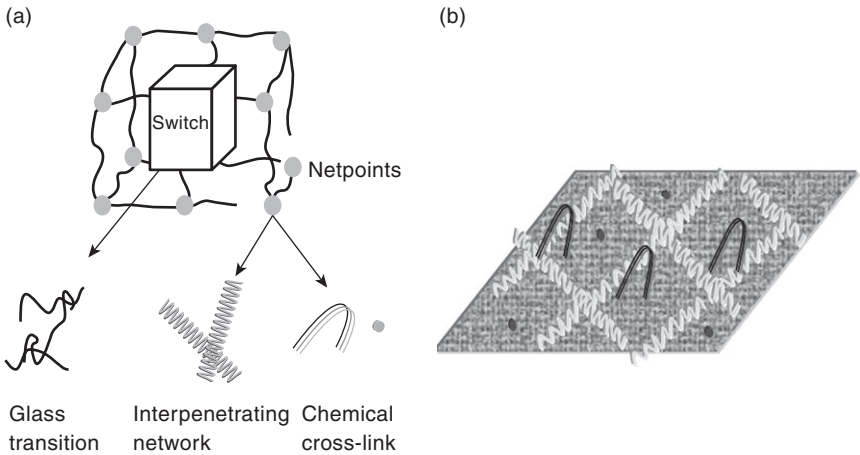
14.2.1 Smart polymer solutions

Smart polymer solutions include water-borne emulsion and microcapsule dispersions in liquid. SMPs can be made by using water-borne emulsions. The water-borne emulsion is prepared by solution polymerization as shown in Fig. 14.1.[11] Shape memory polyurethane (SMPU) oligomers are synthesized with poly(propylene glyols) (PPG), 4,4′-Diphenylmethane diisocyanate (MDI) and dimethylolpropionic acid (DMPA). The –NCO is then end-capped with methyl ethyl ketoxime (MEKO). Triethylamine (TEA) is then added to neutralize the free carboxylic groups. Finally, the mixture of acetone with water is added into the reactor to obtain shape memory aqueous emulsions. A finishing method is usually used to transfer the solution form of SMPs to textiles. The aqueous emulsion is used to finish fabrics using the conventional method of padding→pre-drying→curing process. Under a high curing temperature, the isocyanates that are released react to form cross-linking structures that bond the polymer to fabric.

A small amount of SMP is sufficient to transfer shape memory effects (SMEs) to the fabric during the shape memory finishing process. This process is highly efficient. The general mechanism for shape memory finishing



14.1 Synthesis route of end-capped SMPU oligomers.



14.2 Schematic diagram of shape memory treated fabric.

is shown in Fig. 14.2, where the SMP switch-netpoint model (a) is applied to the fabric surface as shown in (b). The cross-linking between the SMP network and the fibre can form the netpoints and the soft segments of SMPs serve as switches. The SME from the SMPs can thus be transferred to the fabric and maintain its durability during washing.

14.2.2 Microcapsules

In smart polymer applications in textiles, PCMs and colour change polymers mostly take the form of microcapsules. A microcapsule is an intermediate state which is added to solutions, fibres, films and nonwovens to incorporated a smart function into the textile. These materials may be incorpated into textiles by printing, coating and dyeing. For printing and coating, the materials are microencapsulated first and then coated or printed onto the fabric surface by common methods such as the pad-dry-cure process.

Microencapsulation of liquids and solids is an innovative micropackaging technology which provides textiles with new properties.[12,13] Microencapsulation involves the production of microcapsules which act as tiny containers of solids. The wall of a microcapsule is less than 2 μm in thickness and 20–40 μm in diameter. These containers release their contents under controlled conditions to meet a specific purpose. The microcapsules are produced by depositing a thin polymer coating on small solid particles or liquid droplets, or on the dispersions of solids into liquids. The core is the active substance and can be discharged by friction, pressure or diffusion through the polymer wall, dissolution of the polymer wall coating or by biodegradation.

PCMs are either in a solid or liquid state during their application. To prevent dissolution while in the liquid state, PCMs are enclosed in small plastic spheres with diameters of only a few micrometers. These microscopic spheres containing PCM are called PCM-microcapsules. Microscopic spheres that contain colour-changing polymers are called photochromic microcapsules or thermochromic microcapsules.

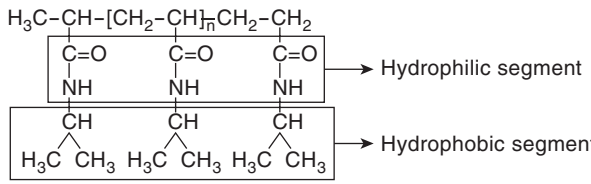
The microencapsulated PCMs are locked into fibres or polyurethane foams by spinning and then finished and coated onto the surface of a textile structure. Polyethylene glycol (PEG) is an important PCM for textile applications. The PCM fibres are then spun using conventional methods such as dry or wet spinning and extrusion of molten polymer. The microencapsulated PCM fibres can store heat for a long time. The composition and properties of a series of sheath/core composite polypropylene nonwoven fibres with different PCM contents have also been studied using scanning electron microscope (SEM), differential scanning calorimetry (DSC) and temperature sensors.[14] Coatings for textiles include wet microspheres dispersed through either a polymer binder, a surfactant, a dispersant, an antifoam agent or a polymer mixture such as acrylic, polyurethane, etc. The coating is applied to a textile substrate by either knife-over-roll, knife-over-air, pad-dry-cure, gravure, dip coating or transfer coating. In order to improve the thermo-physiological wear comfort of garments, PCMs can be incorporated into a thin polymer film and applied to the inner side of fabrics.

14.2.3 Smart polymer gel

Hydrogels are three-dimensional macromolecular gel networks containing a large fraction of liquid within their structures.[15] They can increase or decrease their degree of swelling under specific environmental conditions or stimuli such as temperature, pH or UV light. Hydrophobic interactions among hydrophobic segments will change according to external conditions; so the hydrogen bonding varies from high to low. The final phenomenon is shrinkage or swelling of the hydrogel due to the hydrophobic interactions.

The most studied and used hydrogel in textiles is poly(N-isopropylacrylamide) (PNIPAAm) hydrogel (see Fig. 14.3).[16–22] PNIPAAm hydrogel is a thermal-responsive hydrogel in an aqueous medium around 32–34°C which is close to human body temperature.[23,24] By adjusting copolymer composition and topology, the phase transition can be well controlled.[25,26]

Liu and Hu[17] have reviewed the incorporation of PNIPAAm into textiles to fabricate thermal-responsive hygroscopic fabrics, environmentally sensitive deodorant fibres and stimuli-sensitive nutrient delivery fabrics. Stimuli-responsive hydrogels can be grafted on the surface of cellulose,[27,19,28] polypropylene, polyester and polyamide fabrics[29] using different techniques.



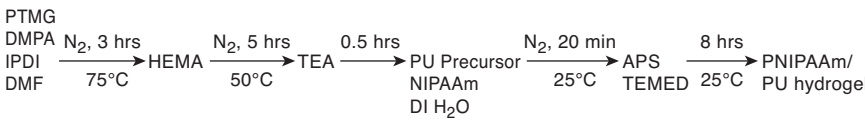
14.3 The molecular structure of PNIPPAm.

These methods include graft polymerization and finishing. Photo-induced graft polymerization was studied by Chen *et al*.[29] Factors affecting the formation of graft polymerization were investigated in terms of the type of additives. The additives ammonium persulphate (APS as initiator), N,N,N′,N′-tetra-methylethylene-diamine (TEMED as promoter), and N,N′-methylene-bisacrylamide (MBAAm as cross-linking agent) were found to be beneficial in promoting the grafting yield. Ammonium cerium nitrate can also be used to initiate the grafting reaction onto the fabric surface and the grafting yield can reach up to 400% using this technology.

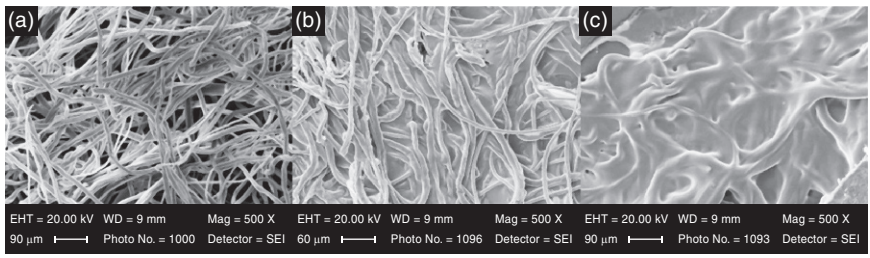
Finishing technology is a simple but versatile and easier method for incorporating hydrogel onto a fabric surface than grafting. Jocic *et al*. researched the chitosan-based hydrogel finishing on polyamide 6,6 fabric surface. The polyamide fabric was immersed in solution and, with 100% pick-up and drying first, was immersed in a pre-gel chitosan solution containing dispersed poly(N-isopropylacrylamide) (PNIPAA) microparticles with a final pick-up of 90%. Finally, the samples were put in the oven for 3 h at 50°C and then rinsed with water under shaking for 24 h. Researchers have developed copolymer P(MEO_2MA-co-OEGMA) composed of 2-(2-methoxyethoxy) ethyl methacrylate (MEO2MA) and oligo(ethylene glycol) methacrylate (OEGMA) as an ideal substitute of PNIPAAm.[30–34]

Durability of the hydrogel on textiles is a critical issue associated with stimuli-responsive hydrogel finished textiles. Jocic[19] proposed two methods to improve the durability of hydrogel on textiles. One is by wetting the textile substrate with the polymer solution, followed by a gelation reaction with the cross-linking solution on the textiles. The other is by spraying aerosol cross-linking solution onto the textile substrate to form a thin layer of hydrogels over the substrate by a rapid interfacial reaction.

Plasma pre-treatment of the fabric surface is also a commonly used method to increase binding ability between hydrogel and matrix in order to increase graft density. Low temperature plasma develops functional groups on textile surfaces which can form covalent bonding between the textile substrate and stimuli-responsive hydrogel. In addition, the sputtering effect of plasma increases the surface roughness of fibres which can enhance the incorporation of stimuli-responsive hydrogel to the substrate.[20]



14.4 Synthesis of PNIPAAm/polyurethane hydrogel with thermal activity.



14.5 The SEM images of the PNIPAAm/polyurethane hydrogel treated fabrics (a) original cotton fabric (b) thin layer of hydrogel coated cotton fabric (c) thick layer of hydrogel coated fabric.

To improve the mechanical compatibility between the hydrogel and the fabrics, Hu *et al.*[16,35,36] incorporated urethane moieties into PNIPPAm when grafting PNIPPAm onto nonwoven fabrics. Firstly, a polyurethane precursor was synthesized by using poly(oxytetramethylene)glycol (PTMG), 2,2-dimethylol propionic acid (DMPA), and isophorone diisocyanate (IPDI) in dimethyl formamide (DMF).[37] Then hydroxylethyl methacrylate (HEMA) was added to react with residual NCO groups of IPDI. Finally, the carboxylic groups in DMPA were neutralized by using TEA. The polyurethane precursor, N-isopropylacrylamide, TEMED and initiator APS were mixed in distilled water and reacted to obtain thermal-responsive composite hydrogels (as shown in Fig. 14.4). The SEM images of N-isopropylacrylamide (NIPAAm)/polyurethane hydrogel treated nonwoven fabrics are shown in Fig. 14.5. Studies showed that the treated textiles can suitably be applied for controlled release of vitamin C. Similarly, perfumes, vitamins and drugs may also be incorporated in the smart textiles for controlled delivery.

Besides the durability and binding ability, thickness of the hydrogel layer is another issue which significantly affects the design of the stimuli-responsive textiles. The thickness of the stimuli-responsive hydrogel coating produced by photopolymerization or plasma treatment polymerization is usually very thin. The thin layer limits the applications of the coated textiles to those such as wound dressings and cosmetics, which require a minimum thickness of hydrogels to take up water, nutrients and drugs, and to

absorb the exudates from wounds. To obtain certain bulky hydrogels, use of cross-linking agents in a solution using free radical polymerization is usually preferred.[38]

14.2.4 Smart polymer films/foams

Film is a common and easily achieved shape for most polymers. Shape memory film or foam has a number of applications in laminated smart fabrics. Various properties of SMP films have been investigated such as thermomechanical properties,[39] different structural factors on the physical and water vapour transport properties,[29,40,41] effects of crystal melting,[40] molecular weight[42] and influence of different processing temperatures.[43]

SMP foams have a variety of applications in textiles such as body fit or damping. A shape memory pillow developed by Bayer can adjust its shape to the contour of the neck and shoulder at around body temperature. SMP foams can be used as memory mattresses to provide body comfort and support. SMP foams have also been used to make insoles, which can effectively improve shoe fitting. Foam is more sensitive for moulding into a body shape which will provide close protection and support as well as a perfect fit for the body.[44,45] With the advantages of impact relaxation and heat insulation of foam, Tobushi *et al*. first prepared and studied SMP foam by chemical foaming in 2001.[46] Similar to the SMP film, strain recovery of the SMP foam took place in the vicinity of T trans. A series of investigations indicated that the holding conditions of strain, temperature and time had great effects on the shape recovery performance of SMP foams[46,47].

Shape memory foams have been used in intimate apparel as temperature-sensitive and shape accommodating behaviour to increase the comfort of the wearer.[48] PCMs were incorporated into polymer film and applied to the inner side of the fabric system by lamination. This laminating method was introduced by Pause.[49] He directly incorporated PCMs into a polymer film (0.3 mm thickness) that was then laminated to the nonwoven fabric system. Pause has claimed that when lamination is compared with the other PCM applications in garments, the method has the following advantages: a high PCM concentration per unit area, low cost of the microencapsulation procedure of the PCM, and minimizing of the weight of the garment.

14.2.5 Smart polymer fibres

Shape memory polymer fibre

To make shape memory polymer fibres, the shape memory polymer needs to have a high molecular weight and suitable viscosity and melting point. The tenacity, elongation, hand-feeling and processability are the main factors

which influence the final end uses. The success of smart polymer fibres has introduced a fibre type to the textiles industry. Commercial PCM fibres and shape memory fibres are being studied.

Hu *et al*.[50–54] developed different SMPU filaments using polyol as the soft segment and small size diols and MDI as the hard segment by wet spinning and melt spinning. Compared to SMPU films, SMPU fibres have lower shape fixity, higher shape recovery and higher recovery stress due to molecular orientation in SMPU fibres brought about by the spinning processes. The recovery ratios of SMPU fibres can be as high as 100%[54] by mixing carbon nanotubes (CNTs) into the SMPU fibres. Hu *et al*.[55] also developed electro-responsive SMP fibres which can recover their shapes under electrical stimulation. The electro-responsive SME is ultimately due to Joule heating of CNTs in SMPU fibres which are electrically charged at both ends. The voltage required to trigger the shape recovery is still very high. For safety reasons, the conductivity of fibres needs to be further improved in order that a low voltage which can trigger the shape recovery can be used.

In fibre form, SMP is more easily applied in textiles. In comparison with shape memory alloy threads, SMP fibres have several advantages like soft feel, no protrusion, larger elongation and greater weaving ability. The SMP fibres have better compatibility with human bodies as a result of their polymeric nature. They can give a look and feel similar to conventional fabrics. SMP fibres are much cheaper than shape memory alloy threads. At present, SMP fibres produced in the lab can have tailorable switch temperature (5–~60°C) and tailorable shape fixity ratios (10–~90%). A plant scale of the SMP fibres is underway.

SMP profiled fibres such as hollow fibres were studied by Meng using melt spinning.[56] The internal diameter of the hollow fibre can noticeably change and recover under thermal stimulation due to SME. Internal diameter change in hollow fibres can affect the physical properties of textile products. Smart SMPU hollow fibres can be used for thermal management garments, or as stuffing in pillows and mattresses, which can adapt to body contours for comfort similar to the function of memory foams.

PCM fibre

The initial fibre with PCM is a hollow fibre filled with PCMs like hollow rayon fibre with $LiNO_3.3H_2O$, Zn $(NO_3)_2.6H_2O.CaCl_2.6H_2O/SrCl_2.6H_2O$ and $Na_2SO_4.10H_2O/NaB_4O_7.10H_2O$ and PEG.[57] It is obvious that the heat capacity decrease of the fibre is greater after more heat–cool cycles and the wash-resistance, durability and handle of the heat-storage textiles produced by these processes are not good.

The PCM fibre spinning process has developed quickly since the 1990s. Magill[58] melted spun polyester (PET) PCM fibres. A composite fibre that

uses PTMG as the core and PET as the sheath was also designed.[59] Zhang *et al*.[60] had studied the melt spin ability of PEG alone, PEG mixed with ethylene-vinyl acetate as the core component, and polypropylene as the sheath by wet and melt spinning.

The fibres made through the microencapsulated PCM composition spinning method also have some disadvantages such as a relatively low latent heat value and very complicated operation. In addition, particle size and size distribution of microencapsulated PCMs also affect the spinnability and mechanical properties of thermo-regulating fibres.[60] This method is restrained in large-scale applications.

Mengjin Jiang[61] studied polyvinyl alcohol (PVA) as fibre matrix, ethyl orthosilicate (TEOS) as membrane-forming reagent and paraffin as PCM to produce thermo-regulating PVA fibre through wet spinning and *in situ* microencapsulation. In this method, it is not necessary to add PCM microcapsules in the spinning dopes, and the advantages of high latent heat, easy operation and low cost are obvious, which will lead to the potential applications of the thermo-regulating fibre or other functional fibres.

Colour change fibre

The fibre method was developed later than the printing and dyeing method for colour change materials. Colour change fibres have better washing durability than solution finishing and film lamination on textiles. The main manufacturing methods of colour change fibres include solution spinning, melt spinning, treatment and graft copolymerization. The polymer and anti-transfer agent are added in the solution and spun directly.

The melt spinning method includes the polymerization, polyblending and sheath-core compound spinning methods. Post-treatment also gives the fibre a chromic property. Common fibres or fabrics are dipped into solution which includes styrene monomer with pyrone, and the fibre and fabrics gain photochromic properties due to the monomer polymerizing. Chromic groups can also be introduced into polymers during polymerization. These polymers can be spun to produce chromic fibres. This method is a polymerization method. The polyblending method uses chromic polymers and polyester, polyamide and other polymers blended and melt spun. It is simple but has a high demand for the chromic polymer. Sheath-core compound spinning is the main method for chromic fibre manufacturing. The core component is a chromic agent and the sheath is a common fibre. The core contains 1–40% chromic agent and the melting point is below 230°C. This chromic fibre has good hand-feeling, washability and colour reaction. Post-treatment and graft copolymerization methods are also used in chromic fibre production. This method is simple and easy to control and extend. The simplest

methods of combining chromic material and fabrics are printing and dyeing. The chromic materials should be encapsulated first and mixed into resin solution. The solution is printed onto the fabric which gains the chromic qualities.[62]

Smart polymer nonwovens with nanofibers

Compared with microfibres, nonwovens with nanofibres were developed in smart polymer textiles such as shape memory polymer nonwovens, PCM nonwovens and colour change nonwovens. SMP electrospun micro- and nanofibre nonwoven structures have unique properties including quicker response to the external stimuli, larger recovery stress, quicker recovery rate and enhanced functionalities such as antibacterial and water vapour permeability.

Nanofibres have been successfully electrospun from SMPU solutions by different people.[63–67] The structures had ultrafine diameters in a range of 50 nm to 2 μm. Uniform nanofibres can be prepared by adjusting applied voltage, concentration and feeding rate. In particular, the concentration plays a key role in controlling its diameters. The cyclic tensile test proves that the resulting nanofibres have good SME: 98% shape recovery and 80% shape fixity can be obtained after several cycles.[63]

The thermal properties of SMPU nanofibrous nonwovens were studied by Zhuo *et al*.[68] and were found to be influenced greatly by the electrospinning and recrystalline conditions. Temperature-strain recovery curves indicate that the SMPU nanofibre tends to have a lower recovery temperature compared with the SMPU bulk film due to their ultrafine diameter. It would be advantageous if material properties could be improved by the construction of microstructures to enhance the shape recovery speed of SMPs without changing the chemical composite. For this purpose, Yang *et al.*[69] reported their work on electrospun micro- to nano-fibre nonwoven films with the diameter of 200 nm to 1 μm. In comparison with the bulk SMPU film, microfibre film has much quicker and sharper shape recovery when heated in water, and the final shape recovery ratio (R_r) and shape fix ratio (R_f) of the microfibre film were also enhanced. DSC and dynamic mechanical analysis (DMA) results indicated that the formation of microfibre has a very limited effect on the switching temperature. The quick shape recovery of the microfibre film is due to the higher surface area of microfibre film which is beneficial for quicker heating/cooling of the sample and quicker diffusion of water. This study offers a possible way to improve the shape recovery speed without changing the chemical composition.[69]

Ozin and co-workers reported another interesting work. The electroactuation of polymer microfibres were spun from an ethoxysilane derivative

of redox active polyferrocenylmethylvinylsilane (PFMVS), and then acid-catalysed condensation of ethoxysilane was performed to get cross-link. In cases of low cross-link density, resultant fibres rapidly respond (<100 ms) to electrical stimuli and large strains occur within 10 ms when fibres are oxidized electrochemically on an electrode surface submerged in a supporting electrolyte.[70]

A novel kind of SMPU nanofibre with core–shell nanostructure was achieved using coaxial electrospinning by Hu and co-workers.[71] In addition to the excellent SME, excellent antibacterial activity against gram-negative and gram-positive bacteria is achieved in the core solution of polycaprolactone based shape memory polyurethane (CLSMPU)–pyridine containing shape memory polyurethane (PySMPU) core–shell nanofibre. It is proposed that the antibacterial mechanism results from the PySMPU shell materials containing the amido group and the high surface area per unit mass of nanofibres. Thus, the core–shell nanofibres can be used as both shape memory and antibacterial nanomaterials.

Recently, with the development of nano-science and technology, ultrafine fibres of PCM/polymer composite[72] have been developed via the electrospinning technique. McCann *et al*.[73] have developed a method based on melt coaxial electrospinning for preparing phase change nanofibres consisting of long-chain hydrocarbon cores and TiO_2–poly(vinyl pyrrolidone) (PVP) sheaths.[73] Chen *et al*. have reported the ultrafine fibres of the polyethylene glycol/cellulose acetate (PEG/CA) composite from the mixture of a solution of PEG and CA by the conventional electrospinning method,[74] and the composite fibres showed a good thermal stability.

14.3 Actuating mechanisms for smart polymers

Such smart functions provided by smart materials are distinct from smart systems or facilities because they do not rely on the complicated sense-response structure of a feedback system. Instead, they can be intrinsically sensitive to changes in their ambient environment, such as temperature, optical wavelength, absorbed gas molecules and pH values.

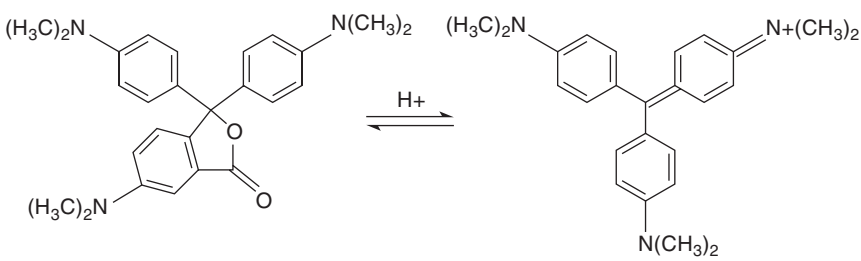
14.3.1 Thermal active

Thermal activity is a widely used stimulus in smart polymer areas and smart textiles. Most SMPs, PCMs, some hydrogels and colour change materials used in textiles are temperature active polymers. Phase change polymers, acting as a kind of thermally active smart polymer, can react immediately to changes in environmental temperatures and body temperatures. When the temperature rises, the PCMs react by absorbing heat and storing energy in

the liquefied form. When the temperature falls, the PCMs release this stored heat energy and become solid again.[2] Thermal-responsive polymeric hydrogels (TRPGs) increase or decrease their degree of swelling below or above a critical temperature, the lower critical solution temperature (LCST)[2,75] or upper critical solution temperature (UCST), respectively.[76]

Thermochromic materials are those whose colour changes when heated, especially thermochromic dyes which can change colour at particular temperatures. Two types of thermochromic organic compounds have been studied and used in textiles: the liquid crystal type and the molecular rearrangement type.[77,78] The molecules of some liquid crystal type thermochromic polymers have helical structures. The length of the helix varies with temperature change, which causes the variations of the light selective reflection and the thermochromic effect. The refractive index and the helical arrangement of the liquid crystal determine the wavelength of the light reflected. Disadvantages of liquid crystal thermochromic material are that it is expensive and sensitive to chemicals which weaken the colour change effects.

Molecular rearrangement based thermochromic materials include spirolactones, fluorans, spiropyrans, and fulgides. These thermochromic materials normally consist of three components: a dye precursor, a colour developer and a non-polar solvent. The colourless dye precursor and colour developer are both microencapsulated.[78] Figure 14.6 shows the rearrangement of the molecular structure of spirolactone, which leads to the reversible thermochromic effect. A proton is donated to the spirolactone by the colour developer to form the dye. Before applying to textiles, thermochromic materials are normally encapsulated.[79,80] Under some temperatures, bisphenol A emits proton, and crystal violet lactone opens rings and combines with the proton to make π system and shows colour. The colour varies with the substituent: when it is H, the colour is violet; when R is CH_3 and X is OCH_3, the colour is blue.



14.6 The rearrangement of spirolactone which leads to thermochromic effect between the colourless dye precursor and coloured dye.

Thermal-active SMPs consist of switch units and netpoints. The earliest reported switches were either amorphous or semi-crystalline phases. During the past decade, researchers have been exploiting more direct switches for SMPs. An amorphous phase with a glass temperature switch, a semi-crystalline phase with a melting temperature switch and even a liquid crystal (LC) phase with an isotropic temperature can be used as switches to make SMPs. Among them, the crystallized phase generally provides the greatest mobility to the entire polymer chain above the melting point. As a result, the T_m-type SMPs possess larger strains. T_g-type SMPs have a higher strength at both low and high temperatures. The LC SMPs are accompanied by a significantly reversible change in polymer chain orientation, allowing the formation of reversible shape changes or two-way SMEs.

SMPs based on glass or melting transition[81] have a physical cross-linking structure, crystalline/amorphous hard phase or chemical cross-linking structure and a low temperature transition of crystalline or amorphous phase. They are processed or thermally set with a desired 'original' shape. Commonly, in the original set shape, internal stress is either zero or very low. If the SMP is subjected to deformation, the applied stress will be stored in the cross-linking structure by cooling the polymer below its switch transition temperature. The deformed temporary shape is thus fixed because of a sharp increase in elastic modulus around the glass or the melting transition temperature. The SMP recovers its permanent shape by heating the polymer above the transition temperature accompanied by the release of the internal stress stored by cross-linked structure. The network to store the internal stress used as a shape recovery driving force may be a physical cross-linking structure, crystalline/amorphous hard phase or chemical cross-linking structure. The 'molecular switch' to resist the release of internal stress can be the crystallization or glass transition. The switching temperature of SMPs is tailorable and can be set around body temperature. The superior processability, soft mechanical properties, high deformability and high recoverability of this kind of SMP makes them suitable for textile applications either by finishing or weaving.

14.3.2 Water/chemical active

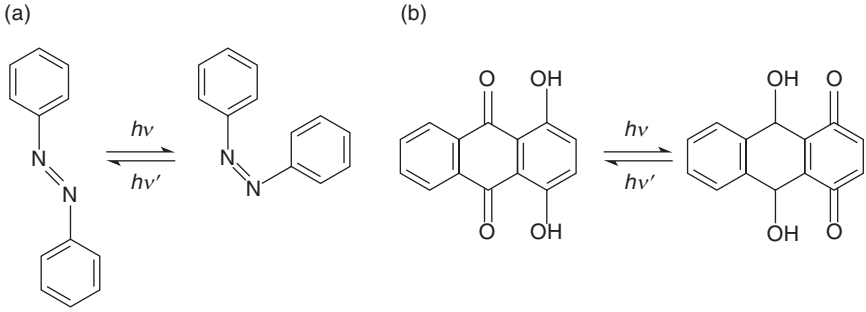
Besides thermally active hydrogels, pH-responsive hydrogels (PRPGs) are also widely studied due to their physiological significance. The pH-responsive hydrogel usually has weak acid or alkaline groups such as carboxyl or amino, respectively.[16,20,82,83] They accept or release protons to change the swelling of the hydrogels in response to pH value. By incorporating the

pH-dependent component acrylic acid into NIPAAm, a thermal/pH dual-responsive hydrogel can be made.[84]

Jocic[21] carried out research to fabricate smart textile surfaces by integrating chitosan hydrogels into textiles. Chitosan (poly(N-acetyl-D-glucosamine-co-D-glucosamine)) is a typical pH-responsive polymer with good biological activity, antibacterial activity, biocompatibility and biodegradability.[85–87] The pH-responsive behaviour of chitosan is due to the high number of amino groups in its chains which can be protonated in different pH solvents.[88,89] When chitosan is under acid or alkali conditions, its molecular chains will be in extended or coiled states, respectively.

SMPs can be triggered by water or moisture due to the plasticizing effect of water molecules, which increases the flexibility of macromolecule chains[90]. If a SMP has a hydrophilic or water soluble ingredient, the shape recovery can be accelerated.[91] Pyridine unit, which is responsive to moisture, can be used to improve the moisture absorption in polyurethane. Hu and coworkers[92] introduced pyridine unit into SMPU by N-bis(2-hydroxylethyl) isonicotinamine (BINA) and prepared moisture-responsive SMPU with high strain recovery and recovery speed. SMPs sensitive to their suitable solvents can be obtained which are similar to hydrophilic SMPs that are sensitive to water/moisture.[93]

In particular, Hu and his team have studied how cellulose nanowhiskers (CNW)/elastomer and CNW/SMPU nanocomposites show novel water sensitivity due to the reversible hydrogen bonding among the –OH groups of the CNWs. A CNW/SMPU nanocomposite with heterogeneous twin switches was synthesized. Interestingly, the resultant CNW/SMPU exhibited triple SMEs upon exposure to sequential thermal and water stimuli.[94] Their investigations showed that incorporating CNWs into crystalline SMPUs enabled the composites not only to maintain a thermally induced SME that originally existed in the polymer matrix, but also to possess a water-induced SME due to the percolation network of the CNWs whose hydrogen bonding can be reversibly regulated by water.[94–96] The ability of CNWs to act as water-sensitive switches provides a new strategy to combine different types of switches into one material by composite methods. Furthermore, the nanocomposites in the dry state exhibited tunable shape recovery, depending on the CNW content, while the thermally induced shape recovery of the composites was found to be negatively proportional to the CNW content.[97] The significance of their work lies in three aspects: 1) the rapid water-responsiveness happens almost instantly, which differs from other methods requiring a number of days for recovery; 2) it is the first example of SM function without using any SMP in the composites; and 3) this water sensitivity is independent of any thermal-responsiveness.



14.7 Different photoisomerization-based photochromisms: (a) photoisomerization of azobenzene and (b) tautomerization of 1,4-dihydroxy anthraquinone.

14.3.3 Light active

Photochromic materials have been widely used in textiles.[98–100] Typical organic photochromic compounds include azobenzenes, spiropyrans, spiroooxazines, viologens, fulgides, 1,4-dihydroxy anthraquinone and diarylethenes. They are based on different photochromisms, that is, isomerization of molecules, ionization of molecules and redox reaction of molecules. Azobenzenes, spiropyrans and viologens have been most widely used in textiles.

The azobenzene photochromism is shown in Fig. 14.7a.[101,102] Figure 14.7b shows the shifting of the hydrogen of 1,4-dihydroxy anthraquinone under the light stimulus, which leads to the colour change of the material. Spiropyrans are the most studied photochromic material based on ionization of molecules. Upon irradiation by light, the covalent bond between the carbon and oxygen breaks and gives ionic pairs. The resulting molecule absorbs photons of visible light and is colourful. When the light is removed, the carbon–oxygen bond reunites and the coloured material recovers its colourless spiropyran. The lifetime of these types of photochromic materials is short because they are susceptible to degradation by oxygen and free radicals. Many studies have been employed to improve their lifetime.[103,104]

Viologens are typical redox reaction photochromic materials. Viologens change colour reversibly upon reduction and oxidation. Other materials with the same photochromism include thiazine derivatives and tetraphernlhydrazine. By copolymerizing light-responsive azobenzene moiety into the TRPG, thermal/light dual-responsive hydrogel can be developed.

14.3.4 Other mechanisms

In electrochromism, a reversible and visible colour change is observed in a material as a result of electrochemical oxidation or reduction. When

oxidation or reduction occurs, the electronic structure of the electrochromic π-conjugated molecule changes, shifting the π–π* electronic absorption. The synthesis of electrochromic polymeric materials has been researched in many laboratories over the last few years. Reynolds and co-workers have successfully developed electrochromic materials with tailored colours.[105] By synthesizing derivatives with a range of band gaps, a broad range of colours has been achieved. Clemson University researchers[106] have prepared bis(3,4-ethylenedioxythiophene)-based materials (BEDOT-Q) for electrochromic applications that provide a broad range of emission wavelengths.

The formation of polyaniline hollow-fibre membranes were studied recently. The potential usefulness of these hollow-fibre membranes is due to the transport of ionic charge carriers to electrochromic coatings. The ionic charge carriers act as dopants to turn the colour on or off.

14.4 The use of smart polymer effects in textiles

Smart polymers exhibit various properties. But how to use these functions in textiles is important for the smart function transformed from polymer to fibres and fabrics. The following contents introduce the functions transformation of smart polymers by various forms such as fibre, solution, film and different processing methods such as spinning, finishing, lamination and coating.

14.4.1 Thermal and moisture management

SMPU polymers for breathable fabrics have a glass transition at around human body temperature. The water vapour permeability (WVP) of the SMPU dense films changes with the wearer's body temperature. When the body temperature is above the glass transition temperature of the polyurethane dense film, the molecular free volume of the film increases significantly and enables the transfer of heat and vapour through perspiration to the environment for comfort. When the body temperature is below the glass transition temperature of the SMPU, the molecular free volume decreases and prevents air and water molecules passing through. Thus, the film can help in maintaining stable body temperature. Employing hydrophilic segments such as dimethylpropionic acid and diol-terminated poly(ethylene oxide)[107,108,109] in SMPUs can improve the overall WVP of dense SMPU films. The overall WVP of SMPs can also be significantly increased by forming micro-foams in the SMPUs. Thermal-responsive SMPU film can be coated, laminated or interlined in conventional fabrics.

Textiles and garments containing PCM are still being developed and investigated. Lamination, coating, knitting and weaving are adopted to

incorporate PCMs into textiles. There are some commercial garments that have microcapsules of PCMs such as the registered mark Outlast®. Outlast Technologies microencapsulated PCMs are called Thermocules™, which can be applied as a finish on fabrics or infused into fibres during the spinning process. The microcapsules are located inside the fibres. The fibres are spun into yarns and then to socks, underwear or knitwear. Outlast® Thermocules® can be coated onto the fabric surface. Nonwovens are coated, and used in jacket linings, but it can also be applied to mid-layers between the first layer and the lining. In this case manufacturers are free to choose any design and first layer they want.

Wang *et al*.[110] reported that one garment providing thermal protection against extreme cold-weather conditions consisted of four layers, of which one layer is a nonwoven polyester fabric treated with PCM enclosed in small polymer spheres with diameters of only a few micrometres. When the temperature of the PCM layer increases above the melting point of the PCM, it melts and becomes liquid. Thermal energy is absorbed and stored during this process. When the temperature of the PCM layer falls, the liquid PCM becomes solid and releases heat energy.

Fibre type and heating rate appeared to have little effect on the overall heat content or thermal performance of the treated fabrics.[57] The PEG-coated fabrics produced by the Mitsui Corporation were used as ski and sportswear.[111] Other thermo-regulated textile products, such as blankets, sleeping bags, underwear, jackets, sports garments, socks, ski boots, helmets, etc., have come into the market since 1997.[73]

14.4.2 Waterproofing and air permeability

Textiles modified with TRPGs displaying swelling/deswelling or hydration/dehydration behaviour will change the water permeability of the textiles. Crespy and Rossi[112] introduced the applications of the thermal-responsive hydrogels in textiles for the purpose of thermal and humidity management of the human body. Kim *et al*.[95] showed that PNIPAAm-grafted polypropylene nonwovens exhibited good thermal-active water permeability. Midé Technology Corporation (USA) used TRPGs as the inner layer of wetsuits to maintain constant temperature in divers by regulating the water flushing permeability of wetsuit fabrics (SmartSkin™).[113,114] In cold diving conditions below the LCST of the hydrogel, the hydrogel swells and reduces water flushing into the wetsuit. Convective heat loss is thus reduced. In warm diving conditions, at temperatures above the LCST of the hydrogel, it shrinks to allow more flushing of water through the wetsuit, increasing the rate of heat loss.

Electrospun SMP nanofibre membranes are good candidates for increasing fabric breathability and enhanced toxic chemical resistance applications. According to Zhuo,[115] the SMPU nanofibrous nonwovens have good liquid and water vapour transfer properties. Moreover, the WVP of the nonwoven is sensitive to changes of relative humidity and temperature (twin-switch). SEM images at a higher temperature show that the porous nanofibrous nonwoven structure is the foundation of unique WVP properties.[115] Similarly, Park and co-workers investigated the applicability of electrospun nanofibre webs as an intelligent clothing material using SMPs. The web, having a high orientation due to an elongation in the process of electrospinning, demonstrated improved shape recovery and good moisture and air permeability due to countless nano-sized pores. Therefore, it can be concluded that the SMPU web has potential for use in intelligent clothing materials.[67]

A porous structure and large surface area to volume ratio endow SMPs with better sensitivity, smart temperature and moisture control. Although research in this area has just started, the outcomes are very inspiring and encouraging. Because of the wide use of SMPs in medical and protective textile materials, the structures could potentially rival existing materials and devices.

SMPU films have become good candidates in breathable laminated nonporous fabrics due to the sensitivity of the WVP to temperature and humidity. In 2000, Jeong *et al*. studied the WVP properties of SMPUs with an amorphous reversible phase and water vapour permeable fabrics by coating the SMPU membranes in a fabric substrate. Similarly, smart WVP was observed around the melting temperature in the SMPUs with a semicrystalline reversible phase. Mondal and Hu studied SMPU-coated fabrics with a tailored transition temperature, that is, room temperature, and studied their WVP properties.[116] The SMPU-coated fabrics showed an abrupt increase in WVP when the temperature reached transition temperature. These results confirm the need for breathable textiles to have a high WVP at higher temperatures and a low WVP at lower temperatures. Additionally, by using SMPUs containing a small percentage of multi-walled carbon nanotubes (MWNTs), Mondal and Hu studied cotton fabric with excellent UV protection, along with a desirable WVP and wearing comfort.[109]

In addition to controlling the WVP by adjusting the temperature, Chen *et al*. adjusted the size and shape of free-volume holes found in membrane materials.[117] Huang *et al*. also studied the influence of hard segment content (HSC) on temperature-sensitive WVP.[118] The influence of hydrophilic groups and crystalline soft segments on the WVP of SMPU film[116] was investigated by Mondal and Hu.[119] It was reported that the WVP increased with the increase of PEG due to the enhanced hydrophilicity. However, the polycaprolactone glycol reduces the WVP in the

polytetramethylene glycol-based SMPU due to the increased interaction among the polymer chains.[116] Mitsubishi Heavy Industries successfully developed 'smart fabrics' that use SMP membranes for the outer wear. The resulting Diaplex™ fabrics show excellent waterproof and breathability properties with 20 000–40 000 mm H_2O in water pressure resistance and 8 000–12 000 g/m^2/24 h in moisture permeability. Such properties provide the resultant garments with high performance and enable wearers to be comfortable in various conditions. In addition, Toray Industries and Marmot Mountain Works® also developed a PU film, MemBrain®. Fabrics laminated with MemBrain® provide a breathable clothing system. By combining the excellent WVP of SMPs with micro-porous coating and laminated technology of garments, SMP coatings and laminates could find a wide application in the textile/garment field, for example, sportswear, footwear, gloves and socks.

14.4.3 Colour change

Chromic textiles responsive to other stimuli such as liquid or gas, electricity, pressure and electron beams have also been invented. These kinds of smart textiles have not been widely used in practice due to the application restrictions.[120]

The commercial thermochromic products include multi-components, namely the colour former, an acidic catalyst and a non-polar co-solvent medium. The organic thermochromic materials are easy to obtain and cheaper than thermochromic liquid crystal, but they are not sensitive and their colour change range is narrow. These materials can be used on some thermochromic printing without high quality demands.

Toray Industries reported the development of a temperature-sensitive fabric using microcapsules of heat sensitive dyes which include four basic colours and 64 combined colours. These are coated homogeneously over fabric surface agents. It can reversibly change colour at temperatures greater than 5°C and is operable from –40°C to 80°C. The change of colour with temperature of these fabrics is designed to match the application.

A kind of solvate chromism fibre was reported,[80] whose colour changes when in contact with a liquid, for example water. These materials are used for 'design' swimsuits. Apart from this, the most important application for chromic materials is to create fantasy designs which change colour depending on the wavelength of incident light.

Photochromic materials are frequently used in Jacquard fabrics, embroidery and prints in different garments for decoration. The Swedish Interactive Institute[121] proposed the use of photochromic fabrics as soft displays. Hallnäs and co-workers dynamically illuminated various parts of

the photochromic fabric to create dynamic textile patterns using a computer-controlled UV lamp.

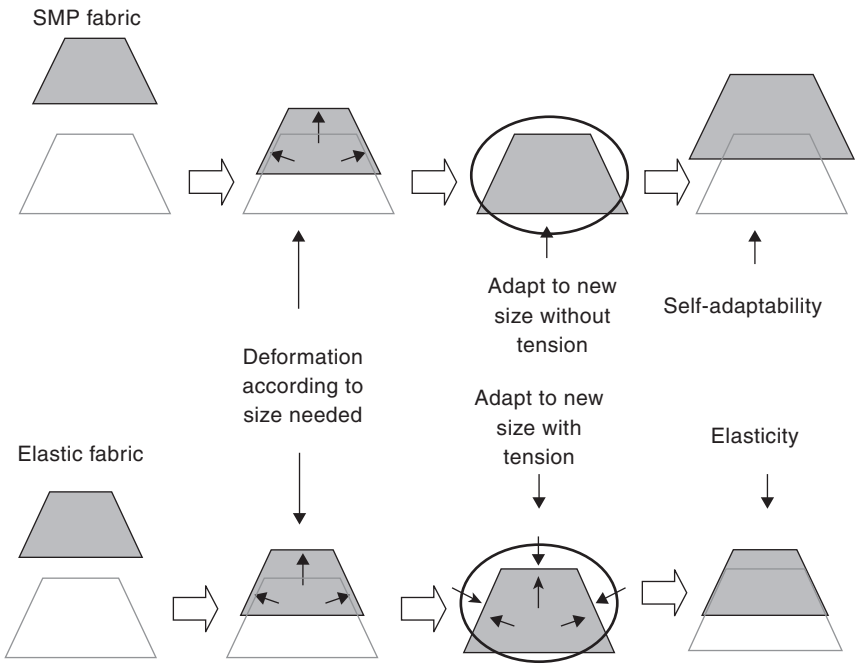
Luminescence differs from chromic effect in that luminescence is not a colour change but emits light. Opticoluminescence is a typical effect encountered in optical fibres. Opticoluminescent fibres have been widely used in textiles for the function of accurately monitoring body and environmental conditions. Recently photonic crystal fibres (PCFs) have been introduced. The cross-section of the PCF contains either periodically arranged micron-scale air voids or a periodic sequence of micron-scale layers of different materials.[122–125] PCFs can appear coloured due to optical interference effects in the microstructured regions.

Red Green Blue (RGB) yarn based on photonic band gap fibres change its emissive colour. The colour of the RGB yarns is very stable over time and largely independent of the fluctuations in the intensity of a light source. The colour changing textiles can be used in uniforms, signage and machine vision which provide imaging-based automatic inspection and analysis for such applications as automatic inspection, process control, and robot guidance in industry.[126,127]

14.4.4 Shape self-adaptability

Shape memory fabrics made of SMP fibres can be used in textiles and clothing to create self-adaptable textiles with self-regulating structures which can perform in response to changes in environmental temperature. Though the SME of the fibre is simply length change, once incorporated into fabrics, this SME can take a variety of forms, such as shrinkage, bending and thickness increase, which is determined by the fabric structures.

Apparel prototypes of shape memory fabrics have been developed incorporating SMP fibres by knitting and weaving.[128,129] The garments made with SMP fibres can be suitably enlarged to fit the body configuration of the wearer.[129] Vertical pressure tests have shown that, in comparison with fabrics made of elastic fibres, the garments made of SMP fibres have a relatively low vertical tension stress. This can be attributed to the deformability and fixability of SMP fibres into temporary shapes, which diminishes the adverse pressure sensation to wearers. As shown in Fig. 14.8, the trapezoids in purple show the garments made of SMP fabrics or elastic fabrics (Spandex fabric). The trapezoids in yellow show the contour of a wearer. The garment made of SMP fibres can enlarge and adapt to the wearer size while no significant pressure is being exerted on the wearer due to the shape fixity of the SMP fibres. The garment made of Spandex fibres generates pressure on the wearer due to the high elasticity of the Spandex fibres in the fabric. The fabric made with SMP fibres with

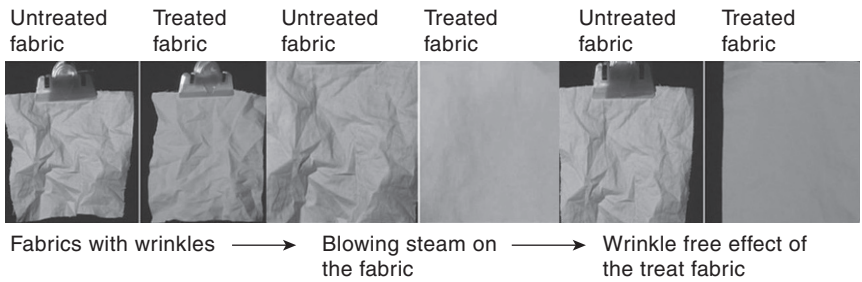


14.8 Self-adaptability of SMP garments adapting to different wearer figures without tension in comparison with garment made of elastic fibres.

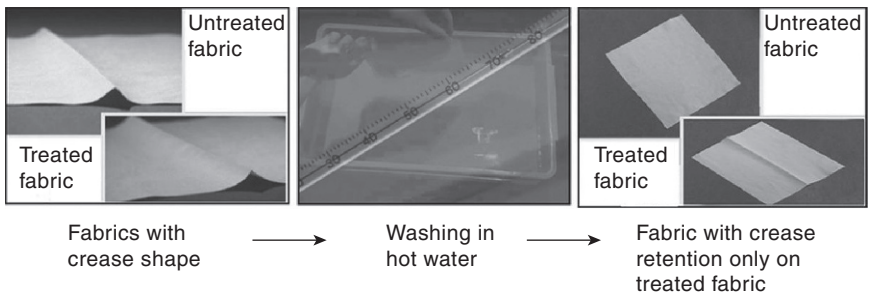
improved comfort sensation can be used especially for intimate apparel and low pressure socks.[128,130]

14.4.5 Shape retention

The SMP treated cotton fabrics have a wrinkle-free effect due to the shape recovery effect of the SMPs.[131] Fabrics, especially cotton materials, develop wrinkles easily under low stress during wearing or storage due to the debonding and slippage of hydrogen bonds. As shown in Fig. 14.9, the cotton fabric treated with SMPU can recover its original flat shape within a minute by blowing steam. However, the unfinished cotton fabric cannot recover its flat appearance because of its low wrinkle resistance. At present, most of the current methods of wrinkle-free finishing use dimethyloldihydroxyethylene urea (DMDHEU) which contains the formaldehyde structure.[132] The advantage of using a SMP emulsion as the finishing agent when compared to DMDHEU is that the treated fabric does not release formaldehyde. Compared with another kind of wrinkle-free finishing by polycarboxylic acid containing finishing agents such as 1,2,3,4-butane tetra-carboxylic acid



14.9 Wrinkle-free effect of the fabric treated with water-borne SMPU in comparison with that of the untreated fabric.

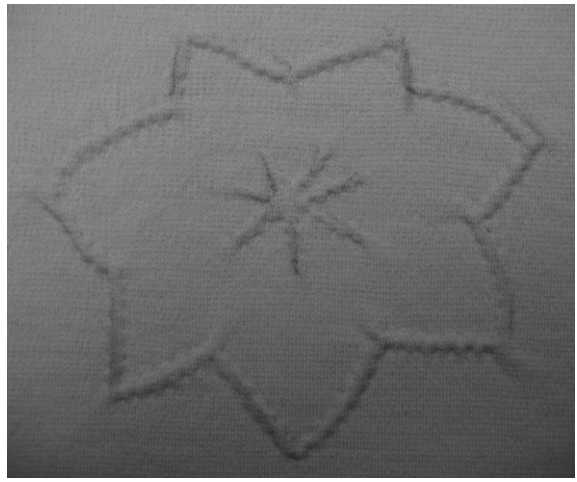


14.10 Crease retention of the fabric treated with SMP compared with that of the untreated fabric (crease shape was produced by ironing).

(BTCA), SMPU finishing does not markedly affect the mechanical strength and whiteness index of the fabric. To some extent SMPU finishing on cotton increases the mechanical strength of fabrics. Repeated washing experiments showed that the wrinkle-free effect of SMPU emulsion treated fabric can last for hundreds of laundering cycles.[133]

Cotton fabrics treated with SMPU emulsion have a greater crease and pattern retention ability due to the excellent shape fixability of SMPs. The crease or pattern design on textiles can add to the aesthetic appeal. Figure 14.10 shows the crease retention effect on cotton fabric treated with SMPs.[134] In Fig. 14.10, both the treated cotton fabric and untreated fabric have a crease shape in the centre set at the beginning by ironing. After hot water washing at a temperature of around 60°C, the crease shape on the untreated fabric disappears while the crease shape on the treated fabric is maintained. Figure 14.11 shows a designed pattern with good pattern retention of a finished knitted fabric.

Wool fabrics were also treated with the SMPU emulsion using a similar reaction mechanism as described previously for cotton fabric. The wool garment treated with SMP emulsion has better dimensional stability than



14.11 The pattern retention of a knitted fabric finished by SMPU after 2 washing cycles.

that of an untreated garment because SMPU covers wool fibre scales and as a result reduces the wool directional frictional effect after the finishing process. The untreated wool garment shrinks to a small size, while the treated garment maintains its size after laundering. In addition, wool fabrics and sweaters have a serious tendency to felting with the entanglement of scales by directional friction. The treated wool fabric has a significantly reduced felting effect when coated with SMPU resin.

14.4.6 Style change for fashion design

Shapes of garments and accessories made of traditional fabric, once the garments and accessories are made, cannot be changed to give other shapes or styles. Though garments made of Spandex fibres can be deformed to any shape, the deformed shape cannot be fixed for aesthetic design because the Spandex fibres shrink to the original length immediately once the external force is released. Shape memory metallic alloy wires can be used in woven fabric for aesthetic design. The garments and accessories made of shape memory metallic alloys can change their shapes with varying environmental and human body temperature. However, there are still many problems associated with the intrinsic properties of metallic alloy. First, because of the significant differences of mechanical and surface properties of shape memory metallic alloy wires and traditional fabrics, shape memory metallic alloy wires have a tendency to protrude out of the fabrics. Complicated structures of fabrics with shape memory metallic alloy wires are difficult to accomplish. Second, due to the low extensibility and high stiffness of shape

memory metallic alloy wires, knitting of shape memory metallic alloy wires is not easy to conduct. Another problem is that if not designed properly, the shape memory metallic alloy wires in shape memory fabrics significantly affect the soft handle of fabrics.

14.5 Using smart polymers in practice: medical textiles

The expanding field of medical textiles comprise all textile products that contribute to improving human health and well-being, protecting us against bacteria and infection, providing external support for injured skin, promoting the healing of wounds and replacing injured and diseased tissues and organs. Smart polymers can provide diversified and multifunctional options for medical textiles' design and fabrication.

14.5.1 Skincare products

The controlled release behaviour and thermally regulated pore size control characteristics of hydrogel modified fabrics may be used for wound dressing, dialysis membranes, drug delivery carriers, separation membranes, skincare and cosmetic materials.[135] With suitable design, the skincare products prepared from stimuli-responsive hydrogel treated textiles can bring a moisturizing, whitening, brightening, or even anti-aging effect to human skin.[136] A facial mask made of the TRPG-treated nonwoven fabric, which is sensitive to the temperature of human skin, can act as a carrier media with controlled release of nutritious ingredients, perfumes or other drugs to human skin in response to changes in human skin temperature.[137] The study has shown that the release of vitamin C could be controlled by varying the surrounding temperature. At present there are still some challenges that need to be solved, such as the high stiffness and brittleness especially when the material is in a dry state.[138]

14.5.2 Wound dressing products

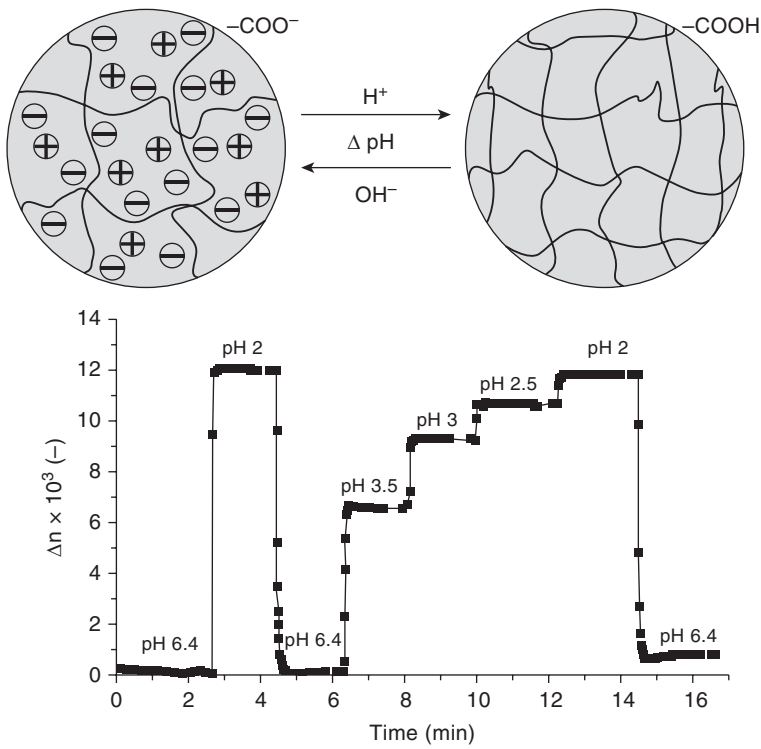
Chitin/chitosan and chitosan derivatives have excellent antibacterial properties and a good wound healing effect.[139] Chitosan hydrogel as a wound dressing can aid in the re-establishment of skin architecture.[140] Alginate filaments and cotton fabric coated with chitosan have been developed for advanced wound dressings.[141] In addition to chitin/chitosan, many hydrogel products for wound dressings have been developed from biopolymers.[142–150] Wound dressings with stimuli-responsive hydrogels can provide a novel drug release system in response to variations in pH/temperature causing the wounds to heal at a faster rate.[151]

14.5.3 Physiological parameter monitoring

pH value is one of the key determinants in controlling the metabolism rate during wound healing and an important parameter for therapeutic interventions in wound care.[152,153] The European Commission supported the project Biotex[154] which developed wound healing monitoring textiles by using pH-sensitive hydrogel polyvinyl acetate (PVA) cross-linked with polyacrylic acid (PAA) using the mechanism shown in Fig. 14.12. Swelling of the pH-responsive hydrogel results in a refractive index change of the hydrogel, and thus can provide information on the stage of the wound healing process.

14.5.4 Deodorant fabrics

Hu and Li[155] disclosed a method to prepare deodorant fabrics which are capable of releasing deodorant agents at certain temperature. The smart fabric is made by coating polymeric hydrogel (N-substituted alkyl) acrylamide, such as N-isopropyl acrylamide on the textile surface. The hydrogel



14.12 pH-sensitive PVA/polyacrylic acid (PAA) hydrogel for wound healing monitoring.

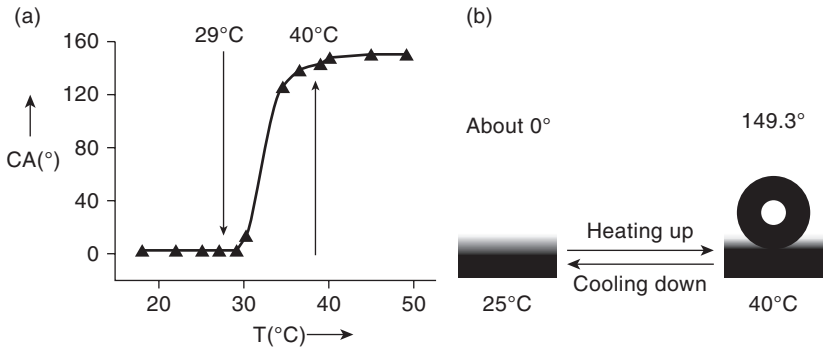
bonds to the textiles by using a functional monomer, such as acrylamide and a cross-linking agent such as 2-(diethylamino) ethyl acrylate. Deodorant can be loaded into the hydrogel during or after the bonding reaction. TRPGs incorporated with β-cyclodextrin (β-CD) can further enhance the controlled release properties of the hydrogel modified textiles.[156] β-CD is a cone-shaped molecule with a hydrophobic interior cavity and a hydrophilic external surface. A variety of hydrophobic guest deodorant molecules can be encapsulated in its cavity.[157]

Some body-responsive deodorant hydrogel products are already available in the market though we do not know exactly what materials have been used because of commercial confidentiality. As far as we know, no deodorant fabric with body-responsive technology has been commercialized yet. For responsive deodorant fabrics, more issues such as soft handle and high stability of the products have to be taken into consideration. Another issue is how to best control the degree of hydration of the product in the open environment, which is affected by many factors.

14.5.5 Reversible superhydrophilic/superhydrophobic fabrics

Smart surfaces with reversible wettability switching between superhydrophobicity and superhydrophilicity are of great importance due to their numerous industrial applications such as in self-cleaning surfaces, microfluidic tools, tunable optical lenses and 'lab-on-chip' systems.[158,159]

The superhydrophobic property of lotus leaves is due to the double structure of the leaves covered in waxes and with a characteristic epidermis possessing numerous papillae at the micro-scale. Wax is hydrophobic and the microstructure enables the superhydrophobicity of lotus leaves. Temperature can change surface chemical compositions of TRPGs and thus can alter the surface wettablity of the surface. Below the LCST, the intermolecular H-bonding between PNIPAAm chains and water molecules is predominant resulting in hydrophilicity. Above the LCST, intramolecular H-bonding in the PNIPAAm chains lead to a compact and collapsed conformation which results in the hydrophobic property.[160] Like lotus leaves, the surface roughness of a TRPG-treated substrate can markedly increase the hydrophobicity and hydrophilicity of the treated surfaces to a very high level. In order to increase surface wetting behaviour, a well-controlled rough silicon surface with groove spacing of about 6 μm was fabricated, on which PNIPAAm films were grafted by Sun *et al*. (see Fig. 14.13).[160] At 40°C, the contact angle was 149.3 ± 2.5°, while at 25°C, the contact angle decreased to about 0°. The high roughness of the substrate is necessary for the reversible switching between the superhydrophilicity and superhydrophobicity on



14.13 (a) The influence of temperature on the contact angle of a PNIPAAm-grafted rough silicon surface. (b) The water drop profile for thermally responsive switching between superhydrophilicity and superhydrophobicity at 25°C and 40°C.

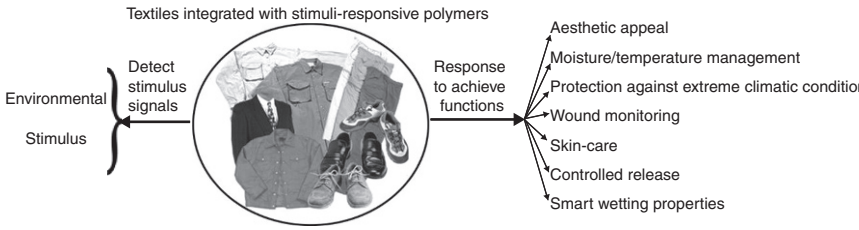
textile surfaces. The microgrooves of about 6 μm in width and about 5 μm in depth in the silicon film described in the reference[160] were generated by a laser cutter on a silicon wafer.

14.6 Conclusion

Smart polymers have been providing enormous opportunities and potential in the textiles industry. By integrating SMPs into textile structures, we can obtain novel functional textiles with profound properties in terms of aesthetic appeal, moisture/temperature management, protection against extreme climatic conditions, textile soft display, wound monitoring, skincare, fantasy design with colour change, controlled release and smart wetting properties on textile surfaces as shown in Fig. 14.14.

Textiles with SMPs can move or change their shapes to achieve different three-dimensional forms in garments, enhancing aesthetic appeal. Window curtains or screens with SRPs can open and close intelligently under environment stimulation. The micro- or macrostructure changes in smart clothing in response to stimuli are a good means for heat and moisture management of human bodies with enhanced comfort. The change of fabric configuration can also be used for protection against extreme environments.

In addition to shape change, stimuli-responsive textiles can offer deformation forces which can be applied for medical applications. Orthopaedic smart textiles can be applied for corrective aids. Furthermore, the textile products developed may be used in clothing for protection against hazardous heat and light. Stimuli-responsive textiles with volume and shape changes may also be used for skincare products with controllable release of perfume, nutrition and drugs. In addition to



14.14 Textiles integrated with SRPs can achieve many novel functions.

the above applications, the stimuli-responsive textiles will open up new opportunities for smart textiles in the field of medicine, widening their applications.

Presently, the study on SRPs for textile applications remains largely unexplored. Even though polymer materials can have good compatibility with textiles, textile applications pose strict requirements on original properties of SRPs such as safety, light weight, high stability, soft handle and good processability. Dyeing, washing and maintenance also need to be taken into consideration for most clothing applications. Much work needs to be carried out to investigate the properties of SRPs in detail and thereby integrate these properties on textile products.

14.7 References

1. Wang Z.L. and Kang Z.C. (1998), *Functional and Smart Materials: Structural Evolution and Structure Analysis*, New York: Plenum Publishing Corp.
2. Yong Q. and Park K. (2001), Environmental-sensitive hydrogels for drug delivery. *Advanced Drug Delivery Reviews*, **53**: pp. 321–329.
3. Vildan Ozturk and Oguz Okay. (2002), Temperature sensitive poly(N-t-butylacrylamide-co-acrylamide) hydrogels: synthesis and swelling behavior. *Polymer*, **43**(18).
4. Yildiz B., Isik B. and Kis M. (2002), Synthesis and characterization of thermoresponsive isopropylacrylamide-acry-lamide hydrogels. *Europe Polymer Journal*, **38**: pp. 1343–1347.
5. Huffman A.S., A.A. and Dong L.C. (1986), Thermally reversible hydrogels: II. Delivery and selective removal of substances from aqueous solutions. *Journal of Controlled Release*, **4**(3): pp. 213–222.
6. Pandaa A.S.B.M., Sabharwalb S., Bhardwajb Y.K. and Majalib A.B. (2000), Synthesis and swelling characteristics of poly (N-isopropylacrylamide) temperature sensitive hydrogels crosslinked by electron beam irradiation. *Radiation Physics and Chemistry*, **58**(1): pp. 101–110.
7. Kanekoa Y., Yoshida R., Sakaia K., Sakuraib Y. and Okano T. (1995), Temperature-responsive shrinking kinetics of poly (N-isopropylacrylamide) copolymer gels with hydrophilic and hydrophobic comonomers. *Journal of Membrane Science*, **101**(1–2): pp. 13–22.

8. Sassi A.P., A.J.S., Han S.M., Blanch H.W. and John M. (1996) Prausnitz, Partitioning of proteins and small biomolecules in temperature- and pH-sensitive hydrogels. *Polymer*, **37**(11): pp. 2151–2164.
9. Bokias G., Staikos, G. and Iliopoulos I. (2000), Solution properties and phase behaviour of copolymers of acrylic acid with N-isopropylacrylamide: the importance of the intrachain hydrogen bonding. *Polymer*, **41**(20): pp. 7399–7405.
10. Zhang Y.-Q., Toyoichi T. and Shibayama M. (1992), Super-absorbency and phase transition of gels in physiological salt solutions. *Nature*, **360**: pp. 142–144.
11. Liu Y.Q., Hu J., Zhu Y. and Yang Z.H. (2005), Surface modification of cotton fabric by grafting of polyurethane. *Carbohydrate Polymers*, **62**: pp. 276–280.
12. Monllor P., Bonet M.A. and Cases F. (2007), Characterization of the behaviour of flavour microcapsules in cotton fabrics. *European Polymer Journal*, **43**(6): pp. 2481–2490.
13. Jahns E. and Burger J. (2005), A step forward in personal comfort: PCM nonwovens. *Nonwovens World*, **14**(1): pp. 51–54.
14. Zhang X.X, Wang X.C., Zhang H. Niu J.J. and Yin R.B. (2003), Effect of phase change material content on properties of heat-storage and thermo-regulated fibres nonwoven. *Indian Journal of Fibre and Textile Research*, **28**(3): pp. 265–269.
15. Osada Y. and Matsuda A. (1995), Shape memory in hydrogels. *Nature*, **376**: pp. 219–220.
16. Liu B, Hu J. and Meng Q. (2009), Nonwoven supported temperature-sensitive poly(N-isopropylacrylamide)/polyurethane copolymer hydrogel with antibacterial activity. *Journal of Biomedical Materials Research: Part B – Applied Biomaterials*, **89B**: pp. 1–8.
17. Liu B. and Hu J. (2005), The Application of Temperature-Sensitive Hydrogels to Textiles: A Review of Chinese and Japanese Investigations Fiber and Textiles in Eastern Europe, **13**: pp. 45–49.
18. Chen K, Ku.Y., Lee C., Lin H., Lin F. and Chen T. (2005), Immobilization of chitosan gel with cross-linking reagent on PNIPAAm gel/PP. *Nonwoven Composites Surface Materials Science and Engineering: C*, **25**: pp. 472–478.
19. Dragon J. (2008), Smart textile materials by surface modification with biopolymeric systems research. *Journal of Textile and Apparel*. **12**: pp. 58–65.
20. Tourrette A., De Geyter N., Jocic D., Morent R., Warmoeskerken M.M.C.G. and Leys C. (2009), Incorporation of poly(N-isopropylacrylamide)/chitosan microgel onto plasma functionalized cotton fibre surface. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **352**: pp. 126–135.
21. Jocic D. (2009), The Perspective of Stimuli-responsive Surface Modifying Systems in Developing Functionalized Textile. in AUTEX World Textile Conference. Izmir, Turkey.
22. Jocic D., Tourrette A., Glampedaki P. and Warmoeskerken M.M.C.G. (2009), Application of temperature and pH responsive microhydrogels for functional finishing of cotton fabric. *Materials Technology*, **24**: pp. 14–23.
23. Pan Y.V., Wesley R.A., Luginbuhi R., Dento D.D. and Ratner B.D. (2001), Plasma polymerized N-isopropylacrylamide: Synthesis and characterization of a smart thermally responsive coating. *Biomacromolecules*, **2**: pp. 32–36.
24. Gupta K.C. and Sahoo S. (2001), Graft copolymerization of acrylonitrile and ethyl methacrylate comonomers on cellulose using ceric ions. *Biomacromolecules*, **2**: pp. 239–247.

25. Suzuki Y., Tomonaga K., Kumazaki M. and Nishio I. (1996), Change in phase transition behavior of an NIPA gel induced by solvent composition: hydrophobic effect. *Polymer Gels and Networks*, **4**: pp. 129–142.
26. Chen G. and Hoffman A. (1995), Graft copolymers that exhibit temperature-induced phase transitions over a wide range of pH. *Nature*, **373**: pp. 49–52.
27. Das M, Zhang H. and Kumacheva E. (2006), Microgels: Old materials with new applications. *Annual Review of Materials Research,* **36**: pp. 117–142.
28. Xie J. and Hsieh Y. (2003), Thermosensitive poly(N-isopropylacrylamide) hydrogels bonded on cellulose supports. *Journal of Applied Polymer Science*, **89**: pp. 999–1006.
29. Chen K.S., Tsai J., Chou C.W., Yang M.R. and Yang J.M. (2002), Effects of additives on the photo-induced grafting polymerization of N-isopropylacrylamide gel onto PET film and PP. *Nonwoven Fabric Surface Materials Science and Engineering: C*, **203–208**.
30. Jonas A.M., Glinel K., Oren R., Nysten B. and Huck W.T.S. (2007), Thermoresponsive polymer brushes with tunable collapse temperatures in the physiological range. *Macromolecules*, **40**: pp. 4403–4405.
31. Lutz J-F, Akdemir Ö. and Hoth A. (2006), Point by point comparison of two thermosensitive polymers exhibiting a similar LCST: Is the age of poly(NIPAM) over? *Journal of the American Chemical Society*, **128**: pp. 13046–13047.
32. Lutz J.F. (2008), Polymerization of oligo(ethylene glycol) (meth)acrylates: Toward new generations of smart biocompatible materials. *Journal of Polymer Science: Part A*, **46**: pp. 3459–3470.
33. Yamamoto S.I., Pietrasik J. and Matyjaszewski K. (2008), The effect of structure on the thermoresponsive nature of well-defined poly(oligo(ethylene oxide) methacrylates) synthesized by ATRP. *Journal of Polymer Science: Part A*, **46**: pp. 194–202.
34. Dong H., Mantha V. and Matyjaszewski K. (2009), Thermally responsive PM(EO)2MA magnetic microgels via activators generated by electron transfer atom transfer radical polymerization in miniemulsion. *Chemistry of Materials*, **21**: pp. 3965–3972.
35. Liu B, Liu W. and Hu J. (2005), Temperature/pH Responsive Composite Nonwoven Fabrics Grafted with Poly(N-isopropylacrylamide)/polyurethane Hydrogel. The Fiber Society 2005 Spring Conference. St. Gallen, Switzerland.
36. Hu J, Liu B. and Liu W. (2006), Temperature sensitive poly(N-isopropylacrylamide)/polyurethane hydrogel modified nonwoven fabrics with controlled release. AATCC International Conference & Exhibition. Boston, USA.
37. Yoshida T., Hattori K., Sawada Y., Choi Y.S. and Uryu T. (1996), Graft copolymerization of methyl methacrylate onto curdlan. *Journal of Polymer Science, Part A: Polymer Chemistry*, **34**: pp. 3053–3060.
38. Park T.G. and Hoffman A. (1994), Deswelling characteristics of poly(N-isopropylacrylamide) hydrogel. *Journal of Applied Polymer Science*, **52**: pp. 85–89.
39. Tobushi, H., *et al*. (1996), Thermomechanical properties in a thin film of shape memory polymer of polyurethane series. *Smart Materials & Structures*, **5**: pp. 483–491.
40. Ding, X.M., Hu J.L. and Tao X.M. (2004) Effect of crystal melting on water vapor permeability of shape-memory polyurethane film, *Textile Research Journal*, **74**(1): pp. 39–43.

41. Jeong, H.M., Ahn B.K., Cho S.M. and Kim B.K. (2000), Water vapor permeability of shape memory polyurethane with amorphous reversible phase. *Journal Of Polymer Science Part B-Polymer Physics*, **38**(23): pp. 3009–3017.
42. Chen, S.J., Hu J., Liem H. Yzhu Y. and Meng Q. (2007), Effect of molecular weight on shape memory behavior in polyurethane films. *Polymer International*, **56**(9): pp. 1128–1134.
43. Hu, J.L., Zeng Y.M. and Yan H.J. (2003), Influence of processing conditions on the microstructure and properties of shape memory polyurethane membranes. *Textile Research Journal*, **73**(2): pp. 172–178.
44. Satoru, K., Shunichi H. and Norio M. (2004) Shoe sock liner.
45. Kazuyuki, K.a.H.S. (1988), Inner boots for ski shoes: Japan.
46. Tobushi, H., *et al.* (2001), Thermomechanical properties of polyurethane-shape memory polymer foam. *Journal Of Intelligent Material Systems And Structures*, **12**(4): pp. 283–287.
47. Tobushi, H., Shimada D., Hayashi S. and Endo M. (2003), Shape fixity and shape recovery of polyurethane shape-memory polymer foams. Proceedings of the Institution of Mechanical Engineers, Part L. *Journal of Materials Design and Applications*, **217**(2): pp. 135–143.
48. Finkelmann, H. and Kundler I. (1998), Director reorientation via stripe-domains in nematic elastomers: influence of cross-link density, anisotropy of the network and smectic clusters. *Macromolecular Chemistry and Physics*, **199**(4): pp. 677–686.
49. Pause, B. (2003), Nonwoven protective garments with thermo-regulating properties. *Journal of Industrial Textiles*, **33**(2): pp. 93–99.
50. Hu J.L., M.Q., Zhu Y., Lu J. and Zhuo H.T. (2007), Shape Memory Fibers Prepared by Wet, Reaction, Dry, Melt, and Electro Spinning in US Patent 11907012: US.
51. Meng Q.H. and Hu J. (2008), Study on poly(ε-caprolactone)-based shape memory copolymer fiber prepared by bulk polymerization and melt spinning. *Polymers for Advanced Technologies*, **19**: pp. 131–136.
52. Hu J.L., M.Q., Zhu Y., Lv J. and Zhuo H.T. 8. (2008), Methods for Preparing Shape Memory Polyurethanes for Textile Products US Patent Office, Pat. No. 61163483.
53. Meng Q.H., Hu J., Zhu Y., Lu J. and Liu Y. (2007), Polycaprolactone-based shape memory segmented polyurethane fiber. *Journal of Applied Polymer Science*, **106**: pp. 2515–2523.
54. Zhu Y., H.J., Yeung L.Y., Liu Y., Ji F.L. and Yeung K.W. (2006), Development of shape memory polyurethane fiber with complete shape recoverability. *Smart Materials and Structures*, **15**: pp. 1385–1394.
55. Meng Q.H., Hu J. and Yeung L.Y. (2007), An electro-active shape memory fibre by incorporating multi-walled carbon nanotubes. *Smart Materials and Structures*, **16**: pp. 830–836.
56. Meng Q., Hu J., Shen L., Hu Y. and Han J. (2009), A smart hollow filament with thermal sensitive internal diameter. *Journal of Applied Polymer Science*, **113**: pp. 2440–2449.
57. Vigo T.L. and Frost C.M. (1983), Temperature adaptable hollow fibers containing polyethylene glycols. *Journal of Coated Fabrics*, **12**(4): pp. 243–254.

58. Magill, C.M., Hartmann, H.M. and Haggard S.J. (2007), Multi-component fibers having enhanced reversible thermal properties and methods of manufacturing thereof, US Patent.
59. Goda and Hironori Matsuyama-shi, J. (2004), Machine crimped synthetic fiber having latent three-dimensional crimpability and method for production thereof.
60. Zhang X.X., Wang X.C. and Tao X.M. (2005), Energy storage polymer/MicroPCMs blended chips and thermo-regulated fibers. *Journal of Materials Science*, **40**: pp. 3729–3734.
61. Jiang M., Song X., Ye G. and Xu J. (2008), Preparation of PVA/paraffin thermal regulating fiber by in situ microencapsulation. *Composites Science and Technology*, **68**(10–11): pp. 2231–2237.
62. Bamfield, P. (2002), *Chromic Phenomena: Technological Application of Colour Chemistry*, Great Britain: Royal Society of Chemistry Ed.
63. Zhuo H.T., Hu J.L. and Chen S.J. (2008), Electrospun polyurethane nanofibres having shape memory effect. *Materials Letters*, **62**(14): pp. 2074–2076.
64. Cha D.I., Kim H.Y., Lee K.H., Jung Y.C., Cho J.W. and Chun B.C. (2005), Electrospun nonwovens of shape-memory polyurethane block copolymers. *Journal of Applied Polymer Science*, **96**(2): pp. 460–465.
65. Zhuo H.T., Hu J., Chen S. and Yeung L. (2008), Preparation of polyurethane nanofibers by electrospinning. *Journal of Applied Polymer Science*, **109**(1): pp. 406–411.
66. Jung Y.C., Kim H.H., Kim Y.A., Kim J.H. (2004), Shape memory polyurethane nanofibers Quality Textiles for Quality Life: pp. 43–46.
67. Chung S.E., Park C.H., Yu W.R. and Kang T.J. (2011), Thermoresponsive shape memory characteristics of polyurethane electrospun web. *Journal of Applied Polymer Science*, **120**(1): pp. 492–500.
68. Zhuo H., Hu J. and Chen S. (2011), Study of the thermal properties of shape memory polyurethane nanofibrous nonwoven. *Journal of Materials Science*, **46**: pp. 3464–3469.
69. Zhang J.-N., Ma Y.M., Zhang J.-J., Xu D., Yang Q.-L., Guan J.-G., Cao X.Y. and Jianga L. (2011), Microfiber SMPU film affords quicker shape recovery than the bulk one. *Materials Letters*, **65**(23–24): pp. 3639–3642.
70. McDowell J.J., Zacharia N.S., Puzzo D., Manners I. and Ozin G.A. (2010), Electroactuation of alkoxysilane-functionalized polyferrocenylsilane microfibers. *Journal of the American Chemical Society*, **132**(10): pp. 3236–3237.
71. Zhuo H.T., Hu J.L. and Chen S.J. (2011), Coaxial electrospun polyurethane core-shell nanofibers for shape memory and antibacterial nanomaterials. *Express Polymer Letters*, **5**(2): pp. 182–187.
72. Chen C., Wang L. and Huang Y. (2008), Morphology and thermal properties of electrospun fatty acids/polyethylene terephthalate composite fibers as novel form-stable phase change materials. *Solar Energy Materials & Solar Cells*, **92**: pp. 1382–1387.
73. Anon (2002), A new class of hydrogels. *Chemical Week*, **164**(22): p. 26.
74. Chen C., Wang, L. and Huang Y. (2008), A novel shape-stabilized PCM: Electrospun ultrafine fibers based on lauric acid/polyethylene terephthalate composite. *Materials Letters*, **62**: pp. 3515–3517.

75. Kopeček, J. (2003), Smart and genetically engineered biomaterials and drug delivery systems. *European Journal of Pharmaceutical Sciences*, **20**: pp. 1–16.
76. Ganji F. and Abdekhodaie M.J. (2010), Chitosan-g-PLGA copolymer as a thermosensitive membrane. *Carbohydrate Polymers*, **80**: pp. 740–746.
77. Carmona N., Herrero-Hernandez E., Llopis J. and Villegas M.A. (2008), Novel sol-gel reversible thermochromic materials for environmental sensors. *Journal of Sol-gel Science and Technology*, **47**: pp. 31–37.
78. Lacasse K. and Baumann W. (2004), *Textile Chemicals Environmental Data and Facts*, Heidelberg: Springer.
79. Canal C, V.S., Ricard A. and Erra P. (2009), Study of irreversible thermochromic ink application on textiles. *Tekstil*, **58**: pp. 105–111.
80. Oakes J., Batchelor S.N. and Dixon S. (2005), Azo dye location in textiles: a novel UV – Vis approach. *Coloration Technology*, **121**: pp. 237–244.
81. Meng Q. and Hu J. (2009), A review of shape memory polymer composites and blends. *Composites Part A*, **40**: pp. 1661–1672.
82. Mc Gann J.M., Higginbotham C.L., Geever L.M. and Nugent M.J.D. (2009), The synthesis of novel pH-sensitive poly(vinyl alcohol) composite hydrogels using a freeze/thaw process for biomedical applications. *International Journal of Pharmaceutics*, **372**: pp. 154–161.
83. Chen J, Liu M., Liu H, Ma L., Gao C., Zhu S. and Zhang S. (2010), Synthesis and properties of thermo- and pH-sensitive poly(diallyldimethylammonium chloride)/poly(N,N-diethylacrylamide) semi-IPN hydrogel. *Chemical Engineering Journal*, **159**: pp. 247–256.
84. Gras S.L., Mahmud T., Rosengarten G., Mitchell A. and Kalantar-zadeh K. (2007), Intelligent control of surface hydrophobicity. *ChemPhysChem*, **8**: pp. 2036–2050.
85. Liu H., Du Y., Wang X. and Sun L. (2004), Chitosan kills bacteria through cell membrane damage. *International Journal of Food Microbiology*, **95**: pp. 147–155.
86. Je J. and Kim S.K. (2006), Antimicrobial action of novel chitin derivative. *Biochimica et Biophysica Acta*, **1760**: pp. 104–109.
87. Lim S.H. and Hudson S.M. (2003), Review of chitosan and its derivatives as antimicrobial agents and their uses as textile chemicals. *Journal of Macromolecular Science, Part C: Polymer Reviews*, **43**: pp. 223–269.
88. Mao J., Kondu S., Ji H.F. and McShane M.J. (2006), Study of the near-neutral pH-sensitivity of chitosan/gelatin hydrogels by turbidimetry and microcantilever deflection. *Biotechnology and Bioengineering*, **95**: pp. 333–341.
89. Khurma J.R. and Nand A.V. (2008), Temperature and pH sensitive hydrogels composed of chitosan and poly(ethylene glycol). *Polymer Bulletin*, **59**: pp. 805–812.
90. Huang W.M. and Yang B. (2005), Water-driven programmable polyurethane shape memory polymer: demonstration and mechanism. *Applied Physics Letters*, **86**: pp. 114105.
91. Chen M.C., Tsai H., Chang Y., Lai W.Y., Mi F.L., Liu C.T.,Wong H.S. and Sung H.W. (2007), Rapidly self-expandable polymeric stents with a shape-memory property. *Biomacromolecules*, **8**: pp. 2774–2780.
92. Chen S., Hu J., Yuen C. and Chan L. (2009), Novel moisture-sensitive shape memory polyurethanes containing pyridine moieties. *Polymer*, **50**: pp. 4424–4428.

93. Lv H., Leng J., Liu Y. and Du S. (2008), Shape-memory polymer in response to solution. *Advanced Engineering Materials*, **10**: pp. 592–595.
94. Luo H.S., Hu J. and Zhu Y. (2011), Polymeric shape memory nano-composites with heterogeneous-twin-switches. *Macromolecular Chemistry and Physics*, **212**: pp. 1981–1986.
95. Shanmuganathan K., Capadona J.R., Rowan S.J. and Weder C. (2010), Biomimetic mechanically adaptive nanocomposites. *Progress in Polymer Science*, **35**: pp. 212–222.
96. Capadona J.R., Shanmuganandam K., Tyler D.J., Rowan S.J. and Weder C. (2008), Stimuli-responsive polymer nanocomposites inspired by the sea cucumber dermis. *Science*, **319**: pp. 1370–1374.
97. Luo H.S., Hu J. and Zhu Y. (2011), Tunable shape recovery of polymeric nano-composites. *Materials Letters*, **65**: pp. 3583–3585.
98. Little A. and Christie R. (2010), Textile applications of photochromic dyes. Part 1: establishment of a methodology for evaluation of photochromic textiles using traditional colour measurement instrumentation. *Colouration Technology*. **126**: pp. 157–163.
99. Little A. and Christie R. (2010), Textile applications of photochromic dyes. Part 2: Factors affecting the photocoloration of textiles screen-printed with commercial photochromic dyes. *Coloration Technology*, **126**: pp. 164–170.
100. Irie, M. (2000), Photochromism: memories and switches – Introduction. *Chemical Reviews*, **100**: pp. 1683–1684.
101. Zhao X.Y., Wang M. and Liu H.J. (2008), Structure dependence of photochromism of azobenzene-functionalized polythiophene derivatives. *Journal of Applied Polymer Science*, **108**: pp. 863–869.
102. Akiyama H., Tanaka A., Hiramatsu H., Nagasawa J. and Tamaoki N. (2009), Reflection colour changes in cholesteric liquid crystals after the addition and photochemical isomerization of mesogenic azobenzenes tethered to sugar alcohols. *Journal of Materials Chemistry*, **19**: pp. 5956–5963.
103. Emin S.M., Sogoshi N., Nakabayashi S., Fujihara T. and Dushkin C.D. (2009), Kinetics of photochromic induced energy transfer between manganese-doped zinc-selenide quantum dots and spiropyrans. *Journal of Physical Chemistry C*, **113**: pp. 3998–4007.
104. Tomasulo M., Deniz E., Alvarado R.J. and Raymo F.M. (2008), Photoswitchable fluorescent assemblies based on hydrophilic BODIPY-spiropyran conjugates. *Journal of Physical Chemistry C*, **112**: pp. 8038–8045.
105. Mortimer R.J., Reynolds A.L. and John R. (2006), *Electrochromic organic and polymeric materials for display applications*. Elsevier.
106. Eaiprasertsak, K. (2008), Electrochemical synthesis and characterization of polymers from Bis(3,4-ethylenedioxythiophene)-thiophenes (Bedot-ts) and bis(3,4-ethylenedioxythiophene)-Benzenes (bedot-Bs) as optoelectronic materials, Clemson University.
107. Jeong H.M., Ahn B. and Kim B.K. (2000), Temperature sensitive water vapour permeability and shape memory effect of polyurethane with crystalline reversible phase and hydrophilic segments. *Polymer International*, **49**: pp. 1714–1721.
108. Mondal S. and Hu J. (2006), Structural characterization and mass transfer properties of nonporous-segmented polyurethane membrane: influence of the

hydrophilic segment content and soft segment melting temperature. *Journal of Membrane Science*, **276**: pp. 16–22.
109. Mondal S. and Hu J.L. (2007), A novel approach to excellent UV protecting cotton fabric with functionalized MWNT containing water vapor permeable PU coating. *Journal of Applied Polymer Science*, **103**(5): pp. 3370–3376.
110. Ji F.L., Zhu Y., Hu J.L., Liu, Y., Yeung, L.Y., and Ye G.D. (2006), Smart polymer fibers with shape memory effect. *Smart Materials & Structures*, **15**(6): pp. 1547–1554.
111. Lewin M. and Preston J. (1993), Fibers with multifunctional properties: a historic approach Handbook of Fiber Science and Technology, Volume III, High Technology Fibers Part C. Vol. 3: CRC press.
112. Crespy D. and Rossi R. (2007), Temperature-responsive polymers with LCST in the physiological range and their applications in textiles. *Polymer International*, **56**: pp. 1461–1468.
113. Macro, S. (2002), Adaptable skin-hydrogel gives wetsuit protection. *Smart Materials Bulletin*, **8**: pp. 7–8.
114. Lin M., Kumar A., Beard S.J. and Qing X. (2002), Built-in structural diagnostic with the SMART LayerTM and SMART SuitcaseTM. *Smart Materials Bulletin*, **7**: pp. 7–8.
115. Zhuo H., Hu J. and S. Chen (2011), Study of water vapor permeability of shape memory polyurethane nanofibrous nonwovens. *Textile Research Journal*, **81**(9): pp. 883–891.
116. Mondal S. and Hu J.L. (2007), Water vapor permeability of cotton fabrics coated with shape memory polyurethane. *Carbohydrate Polymers*, **67**(3): pp. 282–287.
117. Chen, Y., Liu Y., Fan Y., Li H., Shi B., Zhou H. and Peng B. (2007), The polyurethane membranes with temperature sensitivity for water vapor permeation. *Journal of Membrane Science*, **287**(2): pp. 192–197.
118. Huang H., Zhang D., Wang T.J., Mao Z.P., Yu W.D. and Yan H.J. (2007), Influence of hard segment content on the water vapor permeability of segmented shape memory polyurethane membranes. Proceedings of the 2007 International Conference on Advanced Fibers and Polymer Materials, **1–2**: pp. 546–548.
119. Mondal, S. and Hu J.L. (2006), Segmented shape memory polyurethane and its water vapor transport properties. *Designed Monomers and Polymers*, **9**(6): pp. 527–550.
120. Crano J.C. and Gugliemetti R. (2002), *Organic Photochromic and Thermochromic Compounds, Topics in Applied Chemistry*. New York: Kluwer Academic Publishers.
121. Hallnäs L., Melin L. and Redström J. (2002), Design research program for textiles and computational technology. *Nordic Textile Journal*, **1**: pp. 56–62.
122. Gauvreau B., Guo N., Schicker K., Stoeffler K., Boismenu F., Ajji A., Wingfield R., Dubios C. and Skorobogatiy M. (2008), Color-changing and color-tunable photonic bandgap fiber textiles. *Optics Express*, **16**: pp. 15677–15693.
123. Dupuis A., Guo N., Gauvreau B., Hassani A., Pone E., Boismenu F. and Skorobogatiy M. (2007), Guiding in the visible with 'colorful'solid-core bragg fibers. *Optics Letters*, **32**: pp. 2882–2884.

124. Skorobogatiy M. (2009), Microstructured and photonic bandgap fibers for applications in the resonant bio- and chemical sensors. *Journal of Sensors*, **2009**: pp. 524237.
125. Shi L., Pottier P., Skorobogatiy M. and Peter Y.A. (2009), Tunable structures comprising two photonic crystal slabs – optical study in view of multi-analyte enhanced detection. *Optics Express*, **17**: pp. 10623–10632.
126. Woo S.-W., Dokko K. and Kanamura K. (2008), Composite electrode composed of bimodal porous carbon and polypyrrole for electrochemical capacitors. *Journal of Power Sources*, **185**: pp. 1589–1593.
127. Handbook, I.S. (1989), International Organization for Standardization; ISO 2076, 12.89., Geneva, Switzerland.
128. Hu J., Zhu Y., Lu J., Yeung L.Y. and Yeung K.W. (2007), Uniqueness of Shape Memory Fibers in Comparison with Existing Man-made Fibers. in The 9th Asian Textile Conference. Federation of Asian Professional Textile Associations. Taiwan.
129. Liu Y., Chung A., Hu J.L. and Lu J. (2007), Shape memory behavior of SMPU knitted fabric. *Journal of Zhejiang University Science A*, **8**: pp. 830–834.
130. Hu J, Zhu Y. and Lu J. (2008), Self Adjustable, Self Repairable Textiles with Shape Memory Fibers: US Patent.
131. Li Y., Chung S.P., Chan L. and Hu J.L. (2004), Characterization of shape memory fabrics. *Textile Asia*, **35**: pp. 32–37.
132. Hassan, M.S. (2009), Crease recovery properties of cotton fabrics modified by urea resins under the effect of gamma irradiation. *Radiation Physics and Chemistry*, **78**: pp. 333–337.
133. Fan H.J., Hu J. and Ji F.L. (2004), Environmental-Benign thermal-sensitive Polyurethane for Textile Finishing22–24 June 2004. in World Textile Conference 4th AUTEX Conference. Roubaix, France.
134. Hu, J. (2007), VCD_ Shape Memory Finishing of the Shape Memory Textile Centre in the Hong Kong Polytechnic University.
135. Mano, J. (2008), Stimuli-responsive polymeric systems for biomedical applications. *Advanced Engineering Materials*, **10**: pp. 515–527.
136. Hu J., Liu W.G. and Liu B. (2010), Fabric-supported Chitosan Modified Temperature Responsive PNIPAAm/PU Hydrogel and the Use Thereof in Preparation of Facial Mask. US Patent.
137. Hu, J. (2010), Research and Scholarly Activities-Smart Fabrics for Magic Cosmetics. Hong Kong: The Hong Kong Polytechnic University.
138. Liu B.H. and Hu J.L. (2006), *A Study of Thermosensitive Poly(N-isopropylacrylamide) Polyurethane Hydrogel Modified Nonwoven Fabrics*, The Hong Kong Polytechnic University: Hong Kong.
139. Rybicki E., F.B., Kozicki M., Jeziorski A. and Jakubic J. (2005), New Generation Therapeutic Dressings on the Basis of Surface Modified Textiles. in 5th International Scientific Conference Medtex 'Textiles for Medicine'. LoDz-Arturowek, Poland.
140. Ribeiro M., Espiga A., Silva D., Baptista P., Henriques J., Ferreira C., Silva J.C., Borges J.P., Pires E., Chaves P. and Corriea I.J. (2009), Development of a new chitosan hydrogel for wound dressing. *Wound Repair and Regeneration*, **17**: pp. 817–824.

141. Tamura H., Tsuruta Y. and Tokura S. (2002), Preparation of chitosan-coated alginate filament. *Materials Science and Engineering*, **20**: pp. 143–147.
142. Blackburn R.S. (2005), *Alginate Fibres in Biodegradable and Sustainable Fibres*. Cambridge: Woodhead Publishing. 89–109.
143. Kennedy J., Knill Charles J. and Thorley M. (2001), *Natural Polymers for Healing Wounds in Recent Advances in Environmentally Compatible Polymers*, ed. P.G. Edited by Kennedy JF, Williams PA, Hatakeyama H., Cambridge: Woodhead Publishing.
144. Lee S, Kim Y., Chong M. and Lee Y. (2004), Preparation and characteristics of hybrid scaffolds composed of β-chitin and collagen. *Biomaterials*, **25**: pp. 2309–2317.
145. Knill C., Kennedy J., Mistry J., Miraftab M., Smart G. and Groocock M. (2004), Alginate fibres modified with unhydrolysed and hydrolysed chitosans for wound dressings. *Carbohydrate Polymers*, **55**: pp. 65–76.
146. Rajendran S. (2006), Contribution of textiles to medical and healthcare products and developing innovative medical devices. *Indian Journal of Fibre and Textile Research*, **31**: pp. 215–229.
147. Petrulyte S. (2008), Advanced textile materials and biopolymers in wound management. *Danish Medical Bulletin*, **55**: pp. 72–77.
148. Peng H.T., Matrineau L. and Hung A. (2008), Hydrogel-elastomer composite biomaterials: 4. Experimental optimization of hydrogel–elastomer composite fibers for use as a wound dressing. *Journal of Materials Science: Materials in Medicine*, **19**: pp. 1803–1813.
149. Balakrishnan B., Mohanty M., Umashankar P.R. and Jayakrishnan A. (2005), Evaluation of an in situ forming hydrogel wound dressing based on oxidized alginate and gelatin. *Biomaterials*, **26**: pp. 6335–6342.
150. Balakrishnan B., Mohanty M., Fernandez A.C., Mohanan P.V. and Jayakrishnan A. (2006), Evaluation of the effect of incorporation of dibutyryl cyclic adenosine monophosphate in an in situ-forming hydrogel wound dressing based on oxidized alginate and gelatin. *Biomaterials*, **27**: pp. 1355–1361.
151. Cornelius VJ, Majcen N., Snowden M.J., Mitchell J.C. and Voncina B. (2007), Preparation of SMART Wound Dressings Based on Colloidal Microgels and Textile Fibres. Smart Materials IV. Edited by Voelcker, Nicolas H., Proceedings of the SPIE. 6413.
152. Schneider L., Korber A., Grabbe S. and Dissemond J. (2007), Influence of pH on wound-healing: a new perspective for wound-therapy? *Archives of Dermatological Research*, **9**: pp. 413–420.
153. Mokry M., Gal P., Vidinsky B., Kusnir J., Dubayova K., Mozes S., Sabo J. (2006), In vivo monitoring the changes of interstitial pH and FAD/NADH ratio by fluorescence spectroscopy in healing skin wounds. *Photochemistry and Photobiology*, **82**: pp. 793–797.
154. Pasche S, Silvia A., Ischer R., Liley M., Luprano J. and Voirin G. (2008), Wearable biosensors for monitoring wound healing. *Advances in Science and Technology*, **57**: pp. 80–87.
155. Hu J, Z.F., Li P. (2005), Methods of Manufacturing Deodorants, and Deodorants Resulting Thereof, US Patent

156. Liu Y., X.F. (2002), Synthesis and characterization of pH-and temperature-sensitive hydrogel of N-isopropylacrylamide/cyclodextrin based copolymer. *Polymer*, **43**: pp. 4997–5003.
157. Szejtli J. (2004), Past, present, and future of cyclodextrin research. *Pure and Applied Chemistry*, **76**: pp. 1825–1846.
158. Xin B. and Hao J. (2010), Reversibly switchable wettability. *Chemical Society Reviews*, **39**: pp. 769–782.
159. Qi K., Daoud W., Xin J.H., Mak C.L., Tang W. and Cheung W.P. (2006), Self-cleaning cotton. *Journal of Materials Chemistry* **16**: pp. 4567–4574.
160. Sun T., Wang G., Feng L., Liu B., Ma Y., Jiang L. and Zhu D. (2004), Reversible switching between superhydrophilicity and superhydrophobicity. *Angewandte Chemie International Edition*, **43**: pp. 357–360.

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Biopolymers for food packaging applications

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Abstract: This chapter reviews the various uses of renewable polymers in food-related applications and explains how the material chemistry and formulation relate to the required end properties. Biopolymers are currently used in food coatings, food packaging materials and encapsulation matrices for functional foods. They provide unique solutions to enhance product shelf-life whilst also reducing the overall carbon footprint related to food packaging.

Key words: biopolymers, food packaging, food coatings, encapsulation, nanotechnology.

15.1 Introduction

Polymers from renewable resources are currently attracting increasing attention as a method for manufacturing environmentally friendly materials. These polymers can be classified into three groups:

1. polymers directly extracted from biomass, such as polysaccharides (including chitosan, starch and cellulose) and proteins (including caseinates, soy protein, whey protein, gluten and zein);
2. synthetic polymers from oil-based or biomass-derived monomers, such as polycaprolactones (PCL), polyvinyl alcohol (PVOH), ethylene-vinyl alcohol (EVOH) copolymers and polylactic acid (PLA) (Arvanitoyannis *et al*., 1997; Haugaard *et al*., 2001; Petersen *et al*., 1999);
3. polymers produced by natural or genetically-modified microorganisms such as polyhydroxyalcanoates (PHAs) or bacterial cellulose (Martínez-Sanz *et al*., 2011, 2012; Plackett and Siro, 2011).

Within food-related applications, these biobased materials are particularly useful in three main areas: food packaging, food coating and edible films for food and encapsulation.

Currently, the most commercially viable materials in food packaging are certain biodegradable polyesters, which can be processed by conventional equipment. These materials are already used in a number of monolayer and multilayer applications in the food packaging field. Amongst the most widely researched thermoplastics, the sustainable biopolymers used in monolayer packaging include starch, PHA and PLA. Starch and PLA biopolymers are potentially the most attractive types of biodegradable material. This is due to the balance of their properties and the fact that they have become commercially available (e.g., through companies such as Novamont and Natureworks, respectively), being produced on a large industrial scale. PLA is of particular interest in food packaging, due to its excellent transparency and relatively good water resistance. The challenge for these specific biomaterials is to improve their barrier and thermal properties so that they perform like polyethylene terephthalate (PET). Other materials extracted from biomass resources, such as proteins (e.g., zein), polysaccharides (e.g., chitosan) and lipids (e.g., waxes), also have excellent potential as gas and aroma barriers. The main drawbacks of these types of materials are their inherently high rigidity and the difficulty of processing them in conventional equipment. A further disadvantage of proteins and polysaccharides is their very strong water sensitivity caused by their hydrophilic character. This leads to a strong plasticization causing the oxygen barrier (effective when in dry state) to deteriorate as the relative humidity and water sorption in the material increase. This low water resistance of proteins and polysaccharides greatly limits their use in food packaging. It would be highly beneficial to reduce the water sensitivity of proteins and polysaccharides, and to enhance the gas barrier properties and overall functionalities of thermoplastic biopolyesters to improve their properties for food packaging applications.

A range of proteins, polysaccharides and lipids are being used in edible films and coatings. A coating is defined as any type of material used for covering foods, with the aim of extending the shelf-life of the product, which does not need to be removed before eating the food. The use of edible films has several aims, most importantly: the restriction of moisture loss, control of gas permeability, control of microbial activity (e.g., chitosan, which acts against microbes), preservation of the structural integrity of the product and the gradual release of enrobed flavours or antioxidants into the food (Baldwin *et al.*, 2012).

Renewable polymers have also been used for encapsulation purposes. Encapsulation has previously been described as a technology to protect sensitive substances against the influences of adverse environments. The term 'microencapsulation' refers to a defined method of wrapping solids, liquids or gases in small capsules, which can release their contents under specific circumstances (Champagne and Fustier, 2007). Such technologies are of significant interest to the pharmaceutical sector (e.g., for drug and

vaccine delivery), but are gaining relevance for the food industry, especially due to their potential for the protection and delivery of bioactives. Mostly, it is food-grade polymers, such as alginate, chitosan, carboxymethyl cellulose (CMC), carrageenan, gelatin and pectin, which have been applied, using various microencapsulation technologies.

This chapter reviews the potential use of proteins, polysaccharides, lipids and biopolyesters as biodegradable matrices for coating, encapsulation and packaging applications.

15.2 Coatings and active coatings in foods

Edible films or coatings are generally defined as continuous matrices that can be prepared from natural and biodegradable food-grade materials (polysaccharides, proteins and lipids) which serve different functions depending on the current features of the specific product and the anticipated properties of the final product (Tharanathan, 2003). The main role of edible coatings is to preserve the high quality of the food product. Edible coatings have actually been used for centuries to prevent moisture, gas or lipid migration, improve food appearance and increase shelf-life.

Potential properties and applications of edible coatings have been extensively reviewed in recent years (Falguera *et al*., 2011; Han, 2005; Park, 1999; Rojas-Graü *et al*., 2009). The increasing interest in edible films and coatings is due to their ability to incorporate a variety of functional ingredients. For example, plasticizers, such as glycerol, acetylated monoglycerides and polyethylene glycol, are often used to modify the mechanical properties of the film or coating. These additives may cause significant changes to the barrier properties of the film; the application of hydrophilic plasticizers usually increases the water vapour permeability of the films (Fabra *et al*., 2010). However, the major advantage of coatings is that they can be used as a vehicle for incorporating natural or chemical active ingredients, such as antioxidants and antimicrobial agents, enzymes or functional ingredients, like probiotics, minerals and vitamins. These ingredients can be consumed with the food, thus enhancing safety, nutritional and sensory attributes (Cha and Chinnan, 2004; Rojas-Graü *et al*., 2009). Edible films can be used as flavour or aroma carriers in addition to providing a barrier to aroma loss (Guilbert, 1986; Fabra *et al*. 2009, 2012; Reineccius 2009). The transfer of aroma compounds into the packaging modifies the organoleptic properties of the food during storage. New research is also being conducted into the possible application of nanoparticles to edible films and coatings.

To fulfil their functions, edible coatings have to be applied to the product and remain on the product during storage but should disintegrate during cooking or consumption of the coated food product. Recently, Pavlath and

Orts (2009) listed the characteristics required for an ideal film or coating, as follows:

- contain no toxic, allergenic or non-digestible components;
- have good adhesion to food surface;
- provide structural stability and prevent mechanical damage during transportation, handling and display;
- prevent loss of components that stabilize aroma, flavour, nutritional and organoleptic characteristics necessary for consumer acceptance without adversely altering the taste or appearance;
- provide semi-permeability to maintain the internal equilibrium involved in aerobic and anaerobic respiration, thus retarding senescence;
- provide microbiological stability;
- maintain and enhance the sensory attributes (taste, appearance, etc.) of the product;
- serve as a carrier of functional additives; and
- be easily manufactured and economically viable.

The simplest way to apply edible coatings is by dipping the product into, or brushing or spraying the product with, a solution containing the film ingredients. Depending on the concentration of the coating solution, the product will absorb an appropriate amount of coating material necessary to form the desired protective layer at the food surface, when dried. However, coatings obtained with a single polymer component are often fragile and brittle. Some plasticizers need to be added to the coating solution to prevent the developing coatings from becoming brittle. If the coatings crack, the movement of various components will increase by orders of magnitude resulting in mass flow instead of diffusion. Plasticizers should be taken into account as part of the required amount of coating if a high content of plasticizer is added. If so, the water vapour barrier and the mechanical properties of coatings will deteriorate. Hence, the plasticizer–polymer ratio is the key to determining the functional properties of coatings (Coupland *et al*., 2000). Some possible food-grade plasticizers are glycerol, mannitol, sorbitol and sucrose.

Thermo-formation is rarely used to create edible films or coatings, because at high temperatures most edible components undergo structural changes. Hydroxypropyl methylcellulose, starch and PLA are the rare exceptions. Any protein containing cysteine, such as gluten, can serve as a useful thermoplastic biomaterial depending on the level of cysteine.

15.2.1 Role of edible coating additives

The globalization of food markets and the increasing demand for ready-to-eat products, especially minimally processed foods or non-thermally

treated products, represents a challenge for the active packaging industry. Active coatings can be used to aid the preservation of perishable foods. Antimicrobial coating can kill or inhibit the growth of microorganisms and thus extend the shelf-life of perishable products and ensure the safety of packaged products (Han, 2000). Antioxidants may also be incorporated into coatings in order to be released into the food to protect it from oxidative degradation. Furthermore, the controlled release of these active compounds incorporated into packaging is needed to enhance the barrier abilities in the production chain (Han, 2000; LaCoste *et al*., 2005).

Antimicrobial agents

Chitosan has shown great potential as an antimicrobial packaging agent to preserve food against a wide variety of microorganisms (Dutta *et al*., 2009; Fernandez-Saiz *et al*., 2009; 2010; Lagaron *et al*., 2012). Several studies have evaluated how effective the antimicrobial activity of chitosan is when incorporated in other polymer matrices, such as starch (Vásconez *et al*., 2009; Zhai *et al*., 2004; Zhong *et al*., 2011). Vásconez *et al*. (2009) observed that chitosan coating, applied to salmon fillet pieces, had greater antimicrobial effectiveness than when chitosan–tapioca starch blends were applied.

Incorporating antimicrobial compounds into edible films or coatings provides a novel way to improve the safety and shelf-life of ready-to-eat foods (Cagri *et al*., 2004). Some of the more commonly used antimicrobials include benzoic acid, sorbic acid, potassium sorbate, propionic acid, lysozyme, lactoferrin, bacteriocins (nisin and pediocin) and plant-derived secondary metabolites, such as essential oils. However, due to consumer health concerns, natural antimicrobials, such as enzymes and bacteriocins, are preferred in edible and biodegradable packaging materials (Appendini and Hotchkiss, 2002; Datta *et al*., 2008; Suppakul *et al*., 2003). Lysozyme is one of the most frequently used antimicrobial enzymes in packaging materials, since it is a naturally occurring enzyme (Datta *et al*., 2008; Han, 2000; Quintavalla and Vicini, 2002). This enzyme is mainly effective against Gram-positive bacteria, including *Listeria monocytogenes*. Nisin, a polypeptide produced by *Lactococcus lactis*, is also commonly used in food coatings and packaging. Many studies have reported that the addition of nisin to packaging or coating materials inhibits the growth of Gram-positive bacteria, such as *L. monocytogenes* or *Lactobacillus helveticus*, and further extends the shelf-life of perishable foods by suppressing the development of spoilage bacteria (Kim *et al*., 2002). Miller and Cutter (2000) suggested that nisin can be incorporated into collagen-based films and still be active against pathogenic and spoilage bacteria after heat treatments and long-term refrigerated storage.

Other studies have shown that the essential oils of oregano, thyme, cinnamon, lemongrass and clove are among the most active against strains of *Escherichia coli* (Dorman and Deans, 2000; Sánchez-González *et al*., 2011; Smith-Palmer *et al*., 1998). Their effectiveness has been widely reported but carvacrol, a major component of the essential oils of oregano and thyme, has been the most researched (Arfa *et al*., 2007; Guarda *et al*., 2011).

Phenolic compounds present in teas (Friedman *et al*., 2005, 2006), in pigmented rice brans (Nam *et al*., 2006) and in most fruits and vegetables (Friedman *et al*., 2003, 2005; Shahidi and Naczk, 2004) have also shown antibacterial effects. Some of these have been incorporated into edible films and coatings (Cagri *et al*., 2004).

Antioxidant-antibrowning agents

Food oxidation is a destructive process causing colour, flavour and nutritional changes as well as the development of off-flavours (Morales-Aizpurúa and Tenuta-Filho, 2005). Although antioxidants can be added to food products, some studies reveal that adding them directly to initial food formulations is not as effective as expected because once these antioxidants complete the equivalent reaction, their protection ceases and oxidation reactions then increase rapidly (Ozkan *et al*., 2007). Antioxidant active coatings have therefore been suggested as a unique option to release antioxidants into some foods, preventing off-flavour production and food oxidation (Javeed Akhtar *et al*., 2010; Jongjareonrak *et al*., 2008; Pereira de Abreu *et al*., 2011; Torres-Arreola *et al*., 2007). Both natural and synthetic antioxidants have been used in the development of active packaging and have produced promising results. Butylated hydroxytoluene (BHT; E-321) is the most commonly used synthetic antioxidant to form packaging films. Huang and Weng (1998), reported the effectiveness of BHT in preventing the oxidation of fish muscle and oil when it was incorporated in polyethylene films. Its incorporation in environmentally friendly coatings or packaging such as gelatin films (Jongjareonrak *et al*., 2008) is now also being researched. Even though BHT and other synthetic antioxidants, such as butylated hydroxyanisole (BHA; E-320), tertiary butylhydroquinone (TBHQ; E-319) and propyl gallate (E-310), are legal food additives, their use is being reduced. Research is focusing instead on natural antioxidants, such as alpha-tocopherol, citric acid or ascorbic acid, which are becoming the main compounds in active films and packages. Xanthan gum coatings, when mixed with alpha-tocopherol, enhanced nutritional quality and improved the surface colour of peeled baby carrots (Mei *et al*., 2002). Coatings of starch-alginate, starch-alginate-tocopherol and starch-alginate-rosemary have been reported to reduce off-flavours in precooked, refrigerated pork chops and beef patties (Handley *et al*., 1996; Hargens-Madsen, *et al*., 1995; Ma-Edmonds *et al*.,

1995). Carrageenan and whey protein coatings containing ascorbic acid and citric acid were found to prolong the shelf-life of apple slices (Lee *et al*., 2003). Chitosan-based coating containing alpha-tocopheryl acetate significantly delayed the colour change of both fresh and frozen strawberries (Han *et al*., 2004). Recently, Jongjareonrak *et al*. (2008) reported that the antioxidative activity of fish skin gelatin films incorporated with BHT or alpha-tocopherol increased markedly the longer they were stored. Though films without antioxidants had some preventive effect on lard oxidation, this was improved after BHT or alpha-tocopherol addition, due to their migration into the meat product.

Nanotechnology in coating edible biopolymers

Nanocomposites are promising to expand the use of edible films and coatings because the addition of nanoparticles can improve their performance (Sinha and Bousmina, 2005). In recent years, various organic formulations, such as microcrystalline cellulose nanofibres and chitosan nanoparticles have been used in biodegradable polymer matrices to enhance polymer performance. Several studies have been developed which incorporate microcrystalline cellulose nanofibres (Azeredo *et al*., 2009; Bilbao-Sáinz *et al*., 2010a; de Moura *et al*., 2011; Dogan and McHugh., 2007; López-Rubio *et al*., 2007; Olabarrieta *et al*., 2006; Pereda *et al*., 2011; Sánchez-Garcia *et al*., 2010; Siddaramaiah, 2012) or chitosan nanoparticles (de Moura *et al*., 2008, 2009) into nanocomposite edible films. Generally, studies on moisture sorption and water vapour permeability reveal that the addition of cellulose nanocrystals reduces the moisture affinity of hydrophilic films, which is very useful for edible packaging applications. Equally, Bilbao-Sáinz *et al*. (2010b) observed that the water barrier properties of hydrophilic films can be improved by the addition of hydrophobic nanoemulsions into the film matrix.

The controlled release of these nanoparticles is important for long-term storage of foods or for imparting specific desirable characteristics, such as flavour, to a food system (Lagaron, 2011; Sorrentino *et al*. 2007). However, the available literature suggests that many uncertainties remain about nanomaterials, including their potential for bioaccumulation and human health risks. More studies are therefore required to ensure that nanomaterials are not a concern to human health.

15.2.2 Applications of edible coatings

Multicomponent edible films and coatings could have innovative applications in the food industry. The barrier properties of these systems strongly

depend upon their structure and chemistry and the interaction between different film components as well as the surrounding environmental conditions (Wu *et al*., 2002).

Edible films are especially difficult to apply when adding hydrophobic materials to wet surfaces. One possible solution to this problem is dual coating. This provides protection against more than one permeate by using different laminate layers. For instance, the wet cut surface of an apple was first coated with alginate, cross-linked via calcium ions, followed by hydrophobic coating with acetylated monoglyceride (Wong *et al*., 1994).

The applications of edible coatings to processed foods are listed below (Baldwin, 2007; Cutter and Summer, 2002; Ustunol, 2009):

- prevention of moisture loss;
- transfer of water vapour between components of different water activity in a heterogeneous food system;
- formation of ice in frozen foods; and
- exposition to oxygen or diffusion of carbon dioxide.

Coatings can also be used to coat frozen meat and seafood (Coma, 2008; Ustunol, 2009). Kilincceker *et al*. (2009) analysed the effect of edible coatings on the quality of frozen fish fillets. Fillets were coated and stored at –18°C for up to 7 months. Coating materials were applied in three different stages: firstly gluten, secondly xanthan gum and finally wheat and corn flours. The coated fillets were fried and analysed for oil absorption and moisture content throughout the storage period. The study concluded that the coating layers on the meat surface provided more resistance against mass transfer during storage.

Several experiments have also been reported concerning the application of coatings on minimally processed or fresh-cut fruits and vegetables (Cerqueira *et al*., 2009; Lima *et al*., 2010; Ribeiro *et al*., 2007; Rojas-Grau *et al*., 2009). Further, coatings can be used as a means for flavour or nutrient incorporation (Reineccius, 2009).

15.3 Micro- and nanoencapsulation in foods

The development of functional foods containing bioactive ingredients is an area of increasing interest not only for the scientific community but also for the food industry. A functional food is any food that may provide a health benefit beyond the nutrients it usually contains. However, both the physiological benefits and the effectiveness in reducing the risk of diseases depend on preserving the bioavailability of the active ingredients. This represents a formidable challenge for scientists, as the functional substances are known

to lose effectiveness during processing, during storage or in the gastrointestinal tract. The development of micro- and nanostructures, which could protect the active ingredients by acting as carriers and delivery vehicles for controlled release, is undoubtedly a plausible option.

Polymers are particularly useful for encapsulation applications. The use of novel micro- and nanoencapsulation techniques can help to address the main problems of developing novel functional foods, as explained below:

- Incompatibility between the food matrix and the functional ingredient: functional ingredients come in a wide variety of molecular forms which, consequently, lead to differences in physicochemical properties. Therefore, different delivery systems are usually needed depending on the specific molecular and physicochemical concerns associated with each nutraceutical or functional component. An edible delivery system must facilitate the incorporation of the ingredient into the food system. When choosing the food vehicle used to add the chosen bioactive, it is important to consider its solubility in the food matrix and its interactions with other ingredients in the food formulation.
- Loss of activity of the functional ingredient during handling, storage, food processing and/or digestion: one of the main challenges for the development of functional foods is to maintain the bioactivity of the ingredient to be added. Many bioactives are unstable and therefore lose their functionality during processing, storage and/or commercialization (or even during the passage through the gastrointestinal tract, as in the case of probiotic bacteria). Irrespective of the form in which they are incorporated, it is essential that they are stabilized prior to their addition to the food, as the bioactivity needs to be maintained so that, after consumption, it can perform its physiological function when delivered to its particular target site within the body.
- Undesirable changes in taste, flavour or textural properties of food upon addition of the functional ingredient: many bioactives have an objectionable taste and odour. Peptides are known for their bitter taste, mineral salts for their metallic tastes and marine oils, rich in omega-3 fatty acids, for fishy taste and odour. The incorporation of bioactives can thus alter the flavour, odour and texture of foods. As consumers accept food products only with good sensory appeal, introducing these bioactives into a range of functional food products must not compromise food quality. In order to mask a bad-tasting ingredient, it is ideal to have submicron (or nano)-sized capsules of the ingredient so that they are not broken up on mastication. The addition of large particles is undesirable in most cases, hence the increasing need to develop micro- and nanoencapsulation technologies for the food sector.

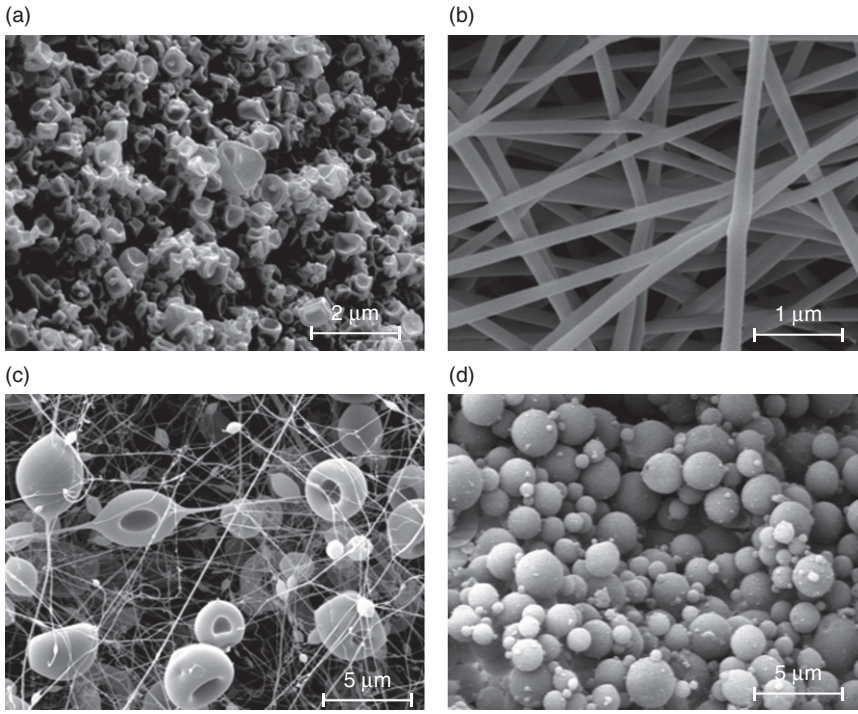
15.3.1 Micro- and nanoencapsulation methods

The choice of encapsulation method depends on the properties of the core and the coating materials, the desired release mechanism, process type, capsule morphology and particle size. Most of these processes have been adapted from the pharmaceutical and chemical industries. The use of low-cost materials and manufacturing processes for encapsulation have posed some difficulties as food products generally have lower profit margins compared with pharmaceutical and chemical products. Both physical (such as spray-drying, spray-chilling or fluidized bed coating) and chemical (such as coacervation, liposome entrapment or inclusion complexation) processes can be used to encapsulate a range of bioactive ingredients (Augustin and Sanguansri, 2008). However, these techniques require heating or the use of organic agents in at least one of the production steps. The former can cause destruction of the sensitive encapsulated nutrients while, if residues of the latter remain, this introduces toxicity problems (Birnbaum *et al*., 2000). Therefore, new technologies which do not involve severe conditions, both in terms of temperature and solvents used, and which give rise to smaller capsule sizes would be highly preferable. The cold gelation technique is an alternative method which does not require the use of organic solvents and requires only mild conditions for capsule formation. This consists of an emulsifying step followed by Ca^{2+}-induced gelation of pre-denatured whey proteins (Beaulieu *et al*., 2002). However, the size of the capsules obtained range from the μm to the mm depending on the calcium concentration (Chen *et al*., 2006). Moreover, most of the technologies described cannot be adapted to a commercial scale.

The electrospinning technique has recently proven to be an efficient alternative method for food products (Lagaron, 2011). Electrospinning is a simple and highly versatile method of producing fibres and/or capsules in the sub-micron range, presenting a large surface to volume ratio. It works by applying an external electric field between two electrodes, imposed on a polymer solution or melt. Therefore, no particular temperature is required in the process. Moreover, although many polymers require organic solvents to dissolve them and employ the electrospinning technique, biopolymers can be electrospun from a watery solution by adjusting the process parameters and/or changing the solution properties through the addition of appropriate supplements. The electrospinning technique has been shown recently to have great potential in the food science area for the development of novel functional ingredients (López-Rubio and Lagaron, 2012; López-Rubio *et al*., 2009, 2012; Torres-Giner *et al*., 2010).

Advantages of the electrospinning technique for encapsulation

One of the main advantages of the electrospinning technique, apart from those already mentioned, is its great versatility in terms of the range of



15.1 Different morphologies of biopolymer-based encapsulation structures obtained through electrospinning. (a) Zein structures; (b) PVOH fibers; (c) pullulan structures; (d) chitosan capsules.

morphologies that can be attained (see for instance Fig. 15.1). The morphology of the structures obtained through electrospinning can be varied by adjusting the process parameters. For a certain material, reduced size capsules can be obtained by lowering the polymer concentration and/or increasing the tip-to-collector distance. When this technique is used, the electrospinning process is normally referred to as 'electrospraying' due to the non-continuous nature of the structures obtained.

Some further benefits that can be obtained through using electrospinning to encapsulate functional ingredients include:

- Easier handling of the nutraceuticals as food ingredients: some successful encapsulation methodologies based on emulsions or gel-particle development have the disadvantage of generating samples in suspension or slurry, which can pose a problem when scaling up technologies for industrial applications.
- Controlled and target delivery of the functional substances: this can be achieved through the rational design and correct encapsulant material

selection. Also, if one wants the ingredient to be released in the stomach or gut, submicron- or nanosized capsules are required so that the capsules are not broken down during the mastication process and the actives are delivered intact to the target area. Hence, the nanometric capsules are of considerable technological importance. The electrospinning technology is able to produce submicron- and nanoparticles which will help with these specific applications.

- Commercially scalable: the electrospinning equipment is available at pilot-plant and even industrial scales, so that large amounts of material can be processed according to the industry requirements. Standard pilot-plant or industrial equipment can be acquired via companies such as BioInicia S.L. (Spain) or ElMarco (Czech Republic), which can adapt it to the requirements for a specific application.

15.3.2 Renewable polymers used for micro- and nanoencapsulation

For food-related applications, encapsulation methods must use natural food components (e.g., proteins, sugars, starches, gums, lipids and cellulosic material) and other ingredients that have 'Generally Recognized As Safe' (GRAS) status (e.g., cyclodextrin, chitosan, low molecular weight emulsifiers such as Tween, mineral salts, etc.). The composition of the components and the choice of encapsulation method define the functional properties of the structures obtained. This limited range of suitable encapsulant materials allowed for food use still remains the biggest challenge to material selection, especially now that food manufacturers and consumers want more sophisticated functional properties. Modification of the existing encapsulant functionality of food-grade materials may be required to achieve new properties in microcapsules and improved performance of microencapsulated ingredients (Augustin and Sanguansri, 2008).

There is now an increased interest in reducing the size of the encapsulates (from micro- to nano-) in order to minimize their possible impact on food texture and appearance. Reducing the size of encapsulates also creates some extra benefits for specific functional food applications, because of the greater specific surface of the particles generated (for instance, improved adhesion properties). Lipids, polysaccharides and proteins have mostly been used to generate nanoencapsulation systems.

Lipid-based nanoencapsulation systems have several advantages, including the ability to entrap material with different solubilities. One of the most researched lipid-based nanocarriers are liposomes. Liposomes are spherical lipid vesicles, which can incorporate a wide range of bioactive compounds

in their hydrophilic interior. They induce stability in water-soluble material, particularly in high-water-activity applications (Gouin, 2004). For food applications, the most important issue is probably the food-grade status of the materials used for making the liposome. Therefore, the study of food-grade liposomes manufactured at the nanoscale level is essential. A particularly useful property of liposomes and nanoliposomes is their ability to incorporate and release two materials with different solubilities simultaneously. This type of nanocarrier has been employed to encapsulate and protect a variety of food bioactives including antioxidants (Takahashi *et al*., 2007) or antimicrobial polypeptides (Were *et al*., 2003).

Proteins also have several advantages for micro- and nanoencapsulation purposes. Given their polyelectrolytic nature, they can be designed for specific delivery applications. A number of factors must be considered when selecting a suitable protein or combination of proteins to fabricate biopolymer-based delivery systems. For instance, it is important to establish the conditions in which the protein molecules are able to associate with other protein or non-protein structure-forming molecules, including the environmental and solution conditions. The important physicochemical characteristics of the proteins, which determine both their molecular conformation and potential interactions with other molecules, include:

- thermal denaturation temperatures for globular proteins;
- helix-coil transition temperatures for gelatin or collagen;
- isoelectric points (pI);
- sensitivities to specific monovalent or multivalent ions; and
- susceptibility to specific enzyme or chemical cross-linking or degradation reactions.

Moreover, it is important to establish the electrical characteristics of the protein molecules involved. Electrostatic interactions are often utilized in structure formation, which can be conveniently described by the z-potential versus pH profile (Matalanis *et al*., 2011). Further, there have been several examples of protein-based encapsulation systems used to stabilize various food bioactives such as probiotics (López-Rubio *et al*., 2012), vitamins (Beaulieu *et al*., 2002) or bioactive peptides (Cho *et al*., 2004).

The third group of materials used for encapsulation is the polysaccharides. These differ from each other chemically in terms of the type, number, sequence and bonding of the monosaccharides within the polymer chain. Their chemical differences, in turn, result in a range of molecular properties, such as molecular weight, degree of branching, structure, flexibility, electrical charge and interactions. These molecular variations determine the different functional properties, such as solubility, thickening, gelation, water-holding capacity, surface activity, emulsification and digestibility (Belitz *et al*., 2009).

A number of factors must be considered when selecting a suitable polysaccharide or combination of polysaccharides to fabricate a biopolymer-based delivery system. It is important to establish suitable environmental and solution conditions in which the polysaccharide molecules can associate with other polysaccharide or non-polysaccharide structure-forming molecules. To do so, one needs to know the physicochemical properties of the polysaccharides involved, such as helix-coil transition temperatures (for carrageenan, alginate, pectin); electrical properties (pKa values); sensitivity to specific monovalent or multivalent ions; or susceptibility to enzyme or chemical reactions (BeMiller and Whistler, 1996). The most widely used carbohydrates for encapsulation purposes are probably alginates (Kailasapathy and Champagne, 2011; Kainmani *et al*., 2011), starch (Li *et al*., 2009) and its linear biopolymer amylose (Lalush *et al*., 2005).

15.3.3 Main encapsulation applications in the food area

Encapsulation of probiotics

A widely accepted definition of probiotics, recognized by the FAO/WHO, states that they are, 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host' (Araya *et al*., 2002). Given this definition, a product containing probiotic organisms should contain a sufficient number of viable cells, proven to be efficacious. This is generally $>10^6$–10^8 CFU/g, or $>10^8$–10^{10} CFU/day.

These bioactive ingredients have been at the forefront of the development of functional foods, particularly in dairy products, and thus deserve particular attention here. In fact, it is estimated that the probiotic industry is responsible for about 10% of the global functional food market (Starling, 2009), which corresponded to €10 billion in 2008. Therefore, products which contain probiotic bacteria are of considerable and growing economic importance. Yet, many studies report that commercial products did not actually contain the stated cell numbers (Lin *et al*., 2006; Weese, 2002) and that some were at unacceptably low levels (Al-Otaibi, 2009; Moreno *et al*., 2006; Shah *et al*., 2000). These data suggest that the levels of probiotic bacteria in commercial products required to have a viable effect pose a problem. They highlight the need to develop efficient technologies to ensure the stability of the probiotic bacteria during commercialization and, ideally, during consumption. A number of methods have been proposed to microencapsulate lactic acid and probiotic bacteria including spray-coating, spray-drying, extrusion, emulsion and gel-particle technologies (which include spray-chilling). Spray-coated products are the most widely used in industry, generating particles between 90 and 250 μm (Champagne *et al*., 2010). However, most of the literature on the microencapsulation of probiotics relates to gel particles or spray-drying

(Chandramouli *et al*., 2004; Doherty *et al*., 2012; Picot and Lacroix, 2003). Although gel particles can be used for this purpose in order to significantly increase viability during storage (Kanmani *et al*., 2011) and resistance to digestion (Doherty *et al*., 2012), the size of the capsules is relatively big (generally >200 μm) and the technology is quite expensive when scaled up for the food industry (Krasaekoopt *et al*., 2003). Microencapsulation by spray-drying is also a well-established process that can produce large amounts of material. While this is an economical and effective technology for protecting materials, it is rarely considered for cell immobilization because of the high mortality resulting from simultaneous dehydration and thermal inactivation of microorganisms.

The most widely used encapsulant material for probiotics is alginate, either alone or in combination with other biomaterials such as chitosan (Kailasapathy and Champagne, 2011; Kainmani *et al*., 2011). Recently, we have developed a proprietary encapsulation technology based on electrospinning that can be successfully applied for protection of probiotics (López-Rubio *et al*., 2009), which is commercially available. This study demonstrated that different food hydrocolloids can be used as encapsulant materials to increase the viability of the bifidobacteria significantly upon storage at various temperatures and even at high relative humidity (López-Rubio *et al*., 2012).

Encapsulation of antioxidants

It has been suggested that the oxidation of biomolecules contributes to several chronic and age-related disorders, including cancer, cardiovascular disease, cataract, diabetes mellitus and rheumatoid arthritis (Willcox *et al*., 2004). Our diet has a huge effect on the production of the antioxidant defence system. Dietary vitamin E, C and β-carotene, as well as other antioxidant plant phenols, including flavonoids, provide essential nutrient antioxidants. However, direct addition of antioxidants to the food products can compromise the maintenance of their bioactivity during long-term storage or during food processing. Moreover, the chemical structure of the antioxidant can sometimes be incompatible with the food matrix. Amongst the broad group of antioxidants, carotenoids and polyphenols have attracted a great deal of research due to the possible health benefits of both groups of compounds (Arts and Hollman, 2005). While endogenous carotenoids in foods are generally stable, when used as food additives they are susceptible to light, oxygen and auto-oxidation. Moreover, dispersion of carotenoids into ingredient systems can cause the product to degrade rapidly (Ribeiro *et al*., 2003). In the case of polyphenols, food processing and storage parameters (e.g., temperature, oxygen and light) as well as the conditions in the gastrointestinal tract (e.g., pH, enzymes and presence of other nutrients) significantly limit

their bioavailability. Further, their usage is limited by their unpleasant taste. A broad range of encapsulation techniques have been applied to antioxidants, such as spray-drying, coacervation, liposomal entrapment, emulsion and co-crystallization (Ribeiro *et al*., 2003). Increased storage stability and improved bioavailability have been observed upon encapsulation using a number of different materials such as starch, maltodextrins, cyclodextrins, alginate or gelatin (Bhandari *et al*., 1999; Sansone *et al*., 2011; Shutava *et al*., 2009; Yu and Huang, 2010). This novel electrospinning technique has also been explored for the protection of various antioxidants (Fernandez *et al*., 2009; López-Rubio and Lagaron, 2012) and the developed structures have proven to control the release properties and improve protection of the bioactivity during simulated *in vitro* digestion (Aceituno, 2011).

Encapsulation of other bioactive ingredients

Currently, the possibility of including a number of other ingredients with potential health benefits in functional foods is being researched. Certain types of peptides have proven to have potential bioactivity, including antioxidant activity, antimicrobial activity, cancer prevention and protection against heart disease (López-Fandiño *et al*., 2006). One of the main drawbacks of these bioactive peptides for direct addition to food products is that they have a bitter or astringent mouthfeel (Cho *et al*., 2004), although this can be prevented through encapsulation. Encapsulation would also be advantageous for omega-3 fatty acids. The growing list of disorders positively affected by dietary omega-3 fatty acids suggests that large portions of the population would benefit from their increased consumption. They are therefore excellent candidates for incorporation into functional foods. The challenges that have to be overcome include the extreme sensitivity of these compounds to oxidative deterioration and the fact that the breakdown products of omega-3 fatty acid degradation can be detected at very early stages of oxidation (Frankel, 2005). Phytosterols are also interesting bioactive compounds. These are plant steroid compounds that have become popular in fortified foods due to their ability to decrease the levels of total and low density cholesterol in humans by inhibiting the absorption of dietary cholesterol (Ostlund, 2004). As with omega-3 fatty acids, encapsulation of phytosterols could increase their oxidative stability.

Controlled release

Proteins, lipids and polysaccharides, either individually or in combination, can be used to create a variety of delivery systems that may be suitable for encapsulating nutraceutical and functional food components. This is usually achieved by starting with a biopolymer solution and then adapting the

environmental conditions to promote the formation of biopolymer particles. The ability of these particles to encapsulate and deliver functional food components depends on their molecular and physicochemical properties, such as composition, internal structure, polarity, electrical charge and physical dimensions (McClements *et al*., 2009). Therefore, it is important to establish the relationship between the characteristics of the molecules present, the nature of the assembly conditions and the final properties of the biopolymer particles formed. Several factors determine the type of interactions taking place between biopolymers: temperature, which promotes conformational changes in proteins and carbohydrates; pH; and ionic strength, which promotes electrostatic interactions. Covalent cross-linking of the biopolymers can also be achieved through a variety of physical, chemical or enzymatic means. Two types of controlled release mechanisms are usually observed during delivery of a bioactive (Lakkis, 2007): (i) delayed release, a mechanism by which the release of a bioactive substance is delayed up to a decided point when/where its release is no longer obstructed (normally triggered by a specific factor) and (ii) sustained release, a mechanism engineered to maintain a constant concentration of a bioactive at its target site. On the other hand, the kinetics of the bioactive release are governed by either one, or a combination of three different mechanisms: diffusion, erosion and swelling.

15.4 Packaging

The most widely researched thermoplastic sustainable biopolymers in monolayer packaging applications are starch, PHAs and PLA. Of these, starch and PLA biopolymers are the most interesting biodegradable materials because they have become commercially available (for example, from companies such as Novamont and Natureworks, respectively), are produced on a large industrial scale and present an interesting balance of properties. PLA is of particular interest in food packaging, due to its excellent transparency and relatively good water resistance. Water permeability of PLA is much lower than in proteins and polysaccharides but is still higher than that of conventional polyolefins and PET. Its relatively high stiffness is usually reduced by the addition of plasticizers but these also lead to a decrease in oxygen barrier and in transparency. The main drawbacks of the performance of this polymer are therefore associated with its low thermal resistance, excessive brittleness and insufficient barrier to oxygen and to water, compared with other benchmark packaging polymers like PET. It is therefore of great industrial interest to enhance the barrier properties of this material while maintaining its inherently good properties, such as its transparency and biodegradability (Bastiolo *et al*., 1992; Chen *et al*., 2003; Jacobsen and

Fritz, 1996; Jacobsen *et al*., 1999; Koening and Huang, 1995; Park *et al*., 2002; Tsuji and Yamada, 2003).

There are other biomaterials with great potential in food packaging applications which are directly extracted from biomass, such as proteins (gluten, zein, etc.) and polysaccharide (i.e., chitosan). Some proteins and polysaccharides have excellent barriers under dry conditions, comparable to EVOH. However, under humid conditions these deteriorate to a much larger extent than EVOH. On the other hand, thermoplastic biopolymers such as PLA or PCL are not as strongly affected by moisture but have lower barriers than the benchmark PET. Research should therefore aim to diminish the water sensitivity of proteins and polysaccharides and to enhance the gas barrier of thermoplastic biopolyesters to make them suitable for monolayer and multilayer food packaging applications.

In summary, the main drawbacks of proteins and polysaccharides are their inherently high rigidity, difficult processability using conventional processing equipment and the acute water sensitivity arising from their hydrophilic character. This last problem leads to a strong plasticization of many properties, including the excellent oxygen barrier, as relative humidity and water sorption increase in the material. The low water resistance of proteins and polysaccharides is a major hindrance to its potential use in monolayers in food packaging.

15.4.1 Novel developments in barrier polymeric structures

There is currently a challenging and broad range of stringent property requirements for polymeric materials. These include:

- ease of processing;
- more effective barrier properties to permanent gases, to moisture and to low molecular weight organic compounds;
- excellent chemical resistance;
- permselectivity;
- low relative humidity dependence for the barrier to be effective; and
- ease of recycling, biodegradability or compostability.

Among the new high barrier polymers currently being developed are the aliphatic polyketones (PK) copolymers (Bonner and Powell, 1997; Lagaron and Powell, 2000). These semicrystalline materials have an outstanding range of mechanical, thermal and high barrier properties (comparable to some EVOH copolymers), as well as chemical resistance and reduced relative humidity dependence for barrier properties. These give them significant

commercial potential in a broad range of engineering, barrier packaging, fibre and blend applications.

Another new range of promising materials currently being investigated with regard to packaging applications are those derived from biomass which, to a varying extent, are easily biodegradable or compostable (Petersen *et al*., 2001; Weber *et al*., 2002). These polymers can have excellent barrier properties to gases. This is the case for plasticized chitosan, although the barrier performance is dramatically reduced in the presence of moisture. However, the PHAs have very high water barrier properties. In principle, one could devise a biomass-derived, high barrier multilayer system, where an inner layer of plasticized chitosan could be sandwiched between high moisture barrier PHA layers. An interesting property of some biobased polymers, such as PLA and starch, is that their permeability to carbon dioxide compared with that to oxygen (permselectivity) is higher than that of most conventional mineral oil-based plastics. This is useful for food packaging applications in which a high barrier to oxygen is required, but CO_2, generated by the product, must be able to leave the package head space to avoid package swelling. Some of these materials, however, are still hindered by high production costs and limitations in certain properties and so cannot compete with other conventional plastic materials now in the market.

There are a significant number of technological developments, using existing materials associated with modern packaging technologies, which aim to tailor designs to produce specific properties and address certain packaged product needs. These developments include (i) multilayer systems comprising various polymeric materials made by lamination, coextrusion or coinjection; (ii) aluminium metallized polymeric films obtained by vacuum deposition technologies; and (iii) oxides (AlO_x or SiO_x) coated with polymeric films. Multilayer systems in the food packaging field usually comprise high gas barrier polymers, like EVOH, between structural layers of other polymers which generally provide a high barrier to moisture as well as other properties like thermal, mechanical, optical or processing properties, printability and thermoweldability. Plastics are coated with vacuum deposited aluminium in order to increase barrier properties to gases, moisture and organic vapours. Further, this results in improved flexibility, consumer appeal and lower environmental impact, due to the corresponding reduction in metal consumption and the fact that the products are more easily recyclable than conventional lamination with aluminium foil. On the other hand, metal coating on polymeric films does reduce flexibility, stretchability and thermoformability compared with the performance of the polymer films alone. Moreover, aluminium coatings render the package opaque and prevent it from being microwaved. To circumvent these problems, polymer films coated with oxides can be made which are transparent and have high barrier properties. The disadvantage is that the coating is susceptible to

microcracking during processing or handling. Aside from coatings, blending with other polymers and nanocomposites is the most commonly considered technology to make more efficient monolayer barrier systems for packaging.

Nanocomposites

Until recently, the main packaging technology based on blending to generate barrier properties was the so-called, 'oxygen scavenger'. This technology aims to ensure that there are relatively low levels of oxygen in contact with the food by trapping permeated oxygen from both the headspace and the outside. However, in carbonated beverages a barrier to carbon dioxide is also a requirement. Most commercial plastic packaging materials, such as PET and its main sustainable counterpart PLA, do not act as a sufficient barrier to these gases. Hence, multilayer structures had to be devised in which one layer (made of EVOH, a crystalline polyamide resin, polyethylenenaphthalate (PEN) or nanocomposites of polyamide 6, PA6) forms a high barrier to carbon dioxide and oxygen while the scavenger reduces oxygen levels at the package headspace. Nanobiocomposites technology can be used to overcome this in monolayer solutions since barrier properties usually correspond not only to oxygen, but also to other gases and low molecular weight components, such as water vapour and food aroma components. Multilayer solutions are currently needed in many food packaging applications such as the aforementioned carbonated beverages. A monolayer solution would also be of great interest for other reasons, including recyclability and the cost of technology and materials.

Nanocomposites technology can also make this possible through simple melt or solution blending routes. In PET bottles, nanocomposites are still manufactured for use in multilayer systems, because the PET nanocomposites are extremely difficult to obtain in a monolayer case owing to the high temperature needed to process the polymer. This temperature breaks down the organophilic chemical modification of the layered clay particles. Some modification of the inorganic layered particles is needed in nanocomposites to aid exfoliation of these into nanolayers during processing and also to enhance compatibility with the polymer. In spite of this, new commercial clay formulations exist that are capable of producing high barrier PET nanocomposites by direct blending, without causing degradation, thereby facilitating monolayer formulations for PET (Sánchez-García *et al.*, 2007).

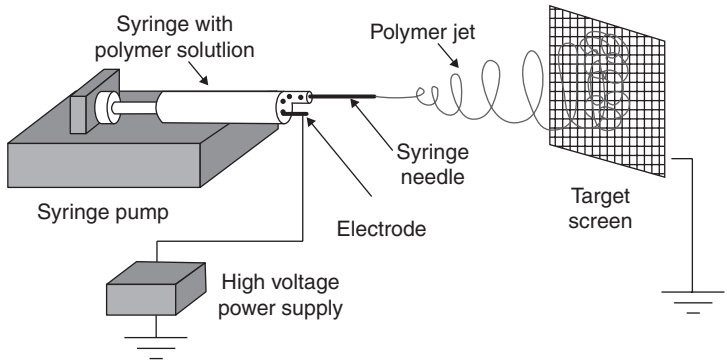
Ultrahigh barrier properties are also needed for food packaging applications in aromatic products, resealable pouches and dehydrated products as well as packaging applications in vacuum and other modified atmospheres. Aluminium, EVOH or other high barrier materials and technologies are

being applied for these, and similar, uses. However, the existing technologies all have certain drawbacks. It is highly desirable to enhance the barrier properties of EVOH three-fold across the whole humidity range, in order to make the material virtually impermeable at dry conditions. It can then form a suitable substitute for typical impermeable materials or technologies, in the many applications where transparency or more simple packaging structures represent added value.

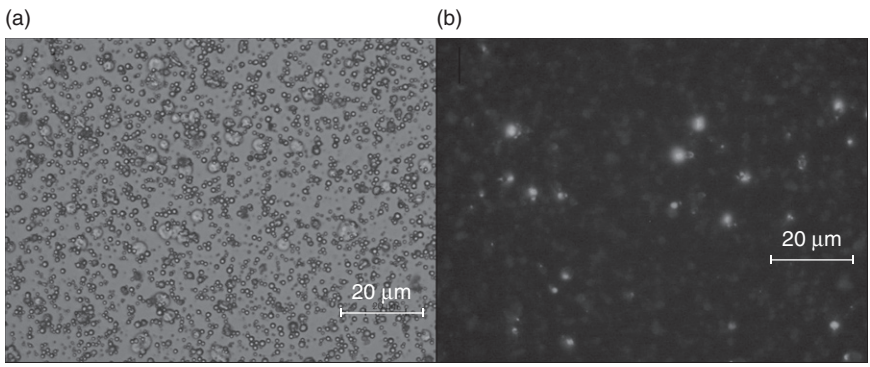
It is of great concern that most of the nanocomposite formulations (first generation nanocomposites) in the market currently use non-food-contact permitted ammonium salts, such as organophilic chemical modifiers, and have been devised to enhance the properties of engineering polymers in structural applications. For food packaging applications only food-contact approved materials and additives should be used, and in quantities below their corresponding threshold migration levels. Only the so-called second generation nanocomposites claim to comply with the current food-contact legislation (Lagaron *et al*., 2006, 2007).

Second generation nanocomposites are referred to as nanocomposite formulations, which are specifically designed to comply with current regulations whilst also being cost effective and formulated to target specific materials (including biopolymers), material properties or production technologies. Second generation nanocomposites are essentially materials with targeted specifications rather than wide spectrum generic formulations. Most applications of nanocomposites in plastics use laminar clays and carbon nanotubes. However, there are other types of reinforcing elements such as biodegradable fibres obtained by electrospinning, which are very promising in a number of application fields (Chronakis, 2005; Huang *et al*., 2003).

The electrospinning method is a simple and versatile technology which can generate ultrafine fibres from many materials, typically in the range 50–500 cm^3. The fibres produced have very large surface area to mass ratios (up to 10^3 higher than a microfibre), excellent mechanical strength, flexibility and lightness. The manufacturing procedure is electrostatic, rather than mechanical, and is applied to the polymer in solution or to polymer melts. The latter method makes it a very suitable technique for the generation of ultrafine fibres of biodegradable materials, which are in general easy to dissolve (Torres-Giner *et al*., 2008). It has been reported that around 100 different polymers (including biopolymers) and polymer blends have been nanofabricated by electrospinning. Figure 15.2 shows a scheme of the electrospinning apparatus. Although there is a significant body of scientific literature reporting the characterization of nanofibres, there are not many studies on the properties of nanocomposites of materials containing these fibres. One of the most interesting aspects of the electrospinning methodology is the potential for the incorporation of various substances, including fillers such as clays, but also of bioactive agents in the electrospun fibres.



15.2 Scheme of electrospinning apparatus.



15.3 Optical micrographs of whey protein concentrate electrosprayed capsules containing encapsulated β-carotene using normal illumination (a) and using a fluorescence source (b).

Figure 15.3 shows optical micrographs of capsules, electrosprayed with whey protein concentrate, containing β-carotene. It can be seen that the antioxidant is properly encapsulated in the matrix.

The advantages of this nanotechnology have already been considered in relation to the controlled release of bioactive principles in the pharmaceutical and biomedical fields and can also be applied to the controlled release of active and bioactive food packaging applications.

Nanotechnology for intelligent packaging

Intelligent packaging is an extension of the communication function of traditional packaging, and imparts information to the consumer based on their ability to sense, detect or record external or internal changes in the product environment. With the move toward globalization, food packaging needs

to allow for longer shelf-life, along with monitoring food safety and quality based on international standards. Simple conventional packing is being replaced with multi-functional intelligent packaging methods to improve food quality, thanks to the application of nanotechnology in this field. New packaging solutions will increasingly focus on food safety by controlling microbial growth, delaying oxidation and improving tamper visibility and convenience. With embedded nanosensors in the packaging, consumers will be able to 'read' the food inside. Sensors can alert us before the food goes rotten or can inform us of the exact nutritional status of the contents.

Multilayered polymers

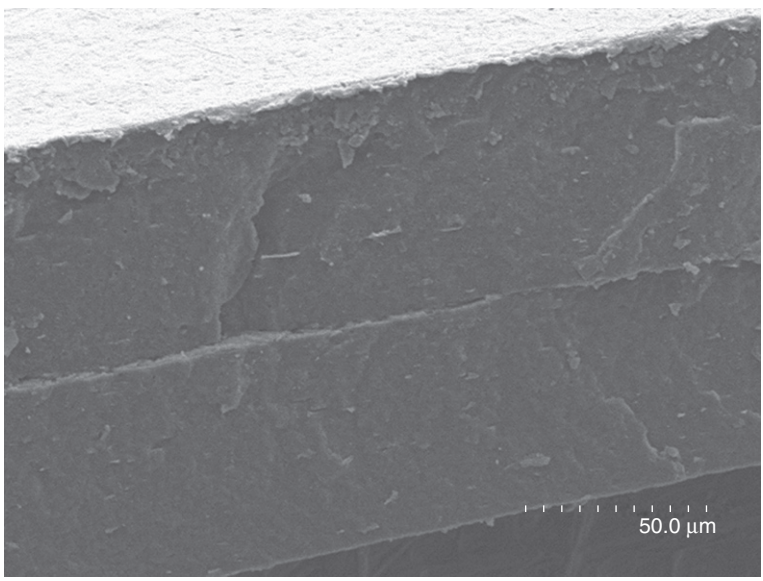
Commercial multilayer polymers currently comprise a number of layers (3–9) of different polymers. Generally, the outer layers consist of polymers, such as polyethylene (PE) or polystyrene (PS), which are inexpensive but with effective water vapour barrier and useful mechanical properties. The barrier layer consists of more expensive materials with good oxygen barrier properties such as PET, EVOH copolymers, polyvinyl alcohol (PVOH), polyamides or polyvinylidene chloride (PVdC) and its copolymers. These plastics are non-renewable and are therefore associated with the greenhouse effect and waste management issues. Environmental concerns have increased demand for novel polymers, mainly those used in food packaging, to be completely renewable and biodegradable. However, biopolymers are still far less effective than the synthetic polymers commonly used meaning that, for certain applications, total replacement is not yet possible. Much research is therefore focused on the improvement of the overall characteristics and, specifically, of the barrier properties of multilayered biodegradable polymers.

One of the key issues in developing a multilayer composite is how to obtain sufficient adhesion between layers. Martin *et al.* (2001) produced multilayer films based on plasticized wheat starch and various biodegradable aliphatic polyesters through flat film co-extrusion and compression-moulding. Different levels of peel strength were found to depend on the compatibility of plasticized starch with the respective polyesters. Polyesteramide was found to have the best adhesion to the plasticized wheat starch layer, probably due to its polar amide groups. Polycaprolactone showed medium adhesion values. PLA and polyhydroxybutyrate-co-valerate (PHBV) were the least compatible polyesters. Coextruded and hot pressed multilayers also demonstrated this trend in adhesion strength. They concluded that it was possible to increase the adhesion properties of the film by up to 50% by introducing either polyester blends in the outer layer or plasticized wheat starch/polyester blends in the inner layer. Rhim *et al.* (2006) reported a multilayer film composed of a soy protein isolate (SPI) inner layer and

PLA outer layers and observed that the mechanical properties of the SPI film were improved by lamination with PLA layers, which were comparable to those of low- and high-density polyethylene (LDPE and HDPE, respectively). More recently, Ghanbarzadeh and Oromiehi (2009) prepared bilayer films based on plasticized whey protein films. They also prepared olive oil-plasticized zein films by casting and subsequently laminated them by compression-moulding and discovered that there was an improvement in tensile properties due to favourable interactions between the layers.

For hydrophilic compounds such as polysaccharides (thermoplastic starch) and proteins, multilayer structures provide certain advantages compared with polymer blends. For instance, moisture sensitivity of these hydrophilic polymers is not completely protected in blends because the phase distribution is close to the surface. However, starch or another hydrophilic film can be held between hydrophobic biodegradable components by using multilayers, thus avoiding the plasticization of the hydrophilic materials.

In our research group, a new method for developing high gas- and vapour barrier multilayers has been developed recently. This links biomass-derived nanocomposites with an electrospinning process, which generates high oxygen barrier structures, even at high relative humidity, by combining layers of biopolyesters with layers based on proteins and polysaccharides. This



15.4 Cross-section of multilayer polyhydroxybutyrate (PHB) systems containing electrospun zein interlayer.

technique is based on creating intermediate or coating electrospun layers of proteins (i.e., zein) or polysaccharides (i.e., pullulan) (see Fig. 15.4) to enhance the barrier properties of other biopolymers whilst also serving as natural tie or adhesive layers (Busolo *et al*., 2009; Fabra *et al*., 2013a, b).

15.5 Conclusion and future trends

It is clear that research into renewable biopolymers is not only demonstrating impressive results at the moment but still has enormous potential in food-related areas. This is particularly the case for the so-called food hydrocolloids and also for the more water resistant, inedible biopolyesters. The former materials have generated more interest in edible applications but also in modern packaging technologies such as biodegradable packaging and active and intelligent packaging. The latter materials are finding significant implementation in structural food packaging. To overcome some of their disadvantageous properties, particularly in terms of their barrier performance, nanotechnology is currently being considered. Equally, the inherent properties of renewable biopolymers are enabling the fast advancement of active and intelligent food packaging and encapsulation technologies.

15.6 References

Aceituno, M. (2011). Desarrollo y caracterización de nanofibras electroestiradas basadas en proteína aislada de amaranto como vehículos para la encapsulación de compuestos antioxidantes. Master thesis supervised by Dr Mendoza and Dr López-Rubio. University of Queretaro (Mexico).

Al-Otaibi, M.M. (2009). Evaluation of some probiotic fermented milk products from Al-Ahsa markets, Saudi Arabia. *American Journal of Food Technology*, **4**, 1–8.

Appendini, P. and Hotchkiss, J.H. (2002). Review of antimicrobial food packaging. *Innovative Food Science and Emerging Technologies*, **3**, 113–126.

Araya, M., Morelli, L., Reid, G., Sanders, M.E. and Stanton, C. (2002). Joint FAO/WHO Working Group Report on Guidelines for the evaluation of probiotics in food, London, Ontario. ftp://ftp.fao.org/es/esn/food/wgreport2.pdf.

Arfa, A.B., Charakabandhu, Y., Preziosi-Belloy, L., Chalier, P. and Gontard, N. (2007). Coating papers with soy protein isolates as inclusion matrix of carvacrol. *Food Research International*, **40**(1), 22–32.

Arts, I.C.W. and Hollman, P.C.H. (2005). Polyphenols and disease risk in epidemiologic studies. *The American Journal of Clinical Nutrition*, **81**, 317s–325s.

Arvanitoyannis, I., Psomiadou, E., Biliaderis, C.G., Ogawa, H., Kawasaki, H. and Nakayama, O. (1997). Biodegradable films made from low density polyethylene (LDPE), ethylene acrylic acid (EAA), polycaprolactone (PLC) and wheat starch for food packaging applications: Part 3. *Starch-Starke*, **49**, 306–322.

Augustin, M.A. and Sanguansri, L. (2008). Encapsulation of bioactives. In: Aguilera J.M., Lillford P.J. (eds). *Food Materials Science: Principles and Practice* (Food Engineering Series). SpringerLink, pp. 577–601.

Azeredo, H.M.C., Mattoso, L.H.C., Wood, D., Williams, T.G., Avena-Bustillos, R.J. and McHugh, T.H. (2009). Nanocomposite edible films from mango puree reinforced with cellulose nanofibers. *Journal of Food Science*, **74**(5), N31–N35.

Azeredo, H.M.C., Miranda, K.W.E., Rosa, M.F., Nascimento, D. M. and de Moura, M.R. (2012). Edible films from alginate-acerola puree reinforced with cellulose whiskers. *LWTFood Science and Technology*, **46**(1), 294–297.

Baldwin, E.A. (2007). Surface treatments and edible coatings. In: M. S. Rahman (Ed.), *Handbook of Food Preservation*, 2nd edn. (pp 477–508). CRC Press, Taylor & Francis, Boca Raton, FL.

Baldwin, E.A., Hagenmainer, R. and Bai, J. (2012). *Edible Coatings and Films to Improve Food Quality*. Taylor & Francis Group, CRC Press, Boca Raton, FL.

Beaulieu, L., Savoie, L., Paquin, P. and Subirade, M. (2002). Elaboration and characterization of whey protein beads by an emulsification/cold gelation process: Application for the protection of retinol. *Biomacromolecules*, **3**, 239–248.

Belitz, H., Grosch, W. and Schieberle, P. (2009). *Food Chemistry*, 4th edn. Springer, Berlin.

BeMiller, J.N. and Whistler, R.L. (1996). Carbohydrates. In O. Fennema (Ed.), *Food Chemistry*. Marcel Dekker, Inc., New York, NY.

Bastiolo, C., Bellotti, V., Del Tredici, G.F., Lombi, R., Montino, A. and Ponti, R. (1992). Complexed starch-containing compositions having high mechanical properties. International Patent Application WO 92/19680.

Bhandari, B.R., D'Arcy, B.D. and Padukka, I. (1999). Encapsulation of lemon oil by paste method using beta-cyclodextrin: Encapsulation efficiency and profile of oil volatiles. *Journal of Agricutural and Food Chemistry* **47**, 5194–5197.

Bilbao-Sáinz, C., Avena-Bustillos, R.J., Wood, D., Williams, T.G. and McHugh, T.H. (2010a). Composite edible films based on hydroxypropyl methylcellulose reinforced with microcrystalline cellulose nanoparticles. *Journal of Agricultural and Food Chemistry*, **58**, 3753–3760.

Birnbaum, D., Kosmala, J., Henthorn, D. and Brannon-Peppas, L. (2000). Controlled release of beta-estradiol from PLAGA microparticles: The effect of organic phase solvent on encapsulation and release. *Journal of Controlled Release*, **65**, 375–387.

Bonner, J.G. and Powell, A.K. (1997). Aliphatic polyketones as high barrier materials, '213th National American Chemical Society Meeting', ACS. Materials Chemistry Publications, Washington.

Busolo, M., Torres-Giner, S. and Lagaron, J.M. (2009). Enhancing the Gas Barrier Properties of Polylactic Acid by Means of Electrospun Ultrathin Zein Fibers. *ANTEC, Proceedings of the 67th Annual Technical Conference*, Chicago, IL, 2763–2768.

Cagri, A., Ustunol, Z. and Ryser, E.T. (2004). Antimicrobial edible films and coatings. *Journal of Food Protection*, **67**, 833–848.

Cerqueira, M.A., Lima, A.M., Teixeira, J.A., Moreira, R.A. and Vicente, A.A. (2009). Suitability of novel galactomannans as edible coatings for tropical fruits. *Journal of Food Engineering*, **94**(3–4), 372–378.

Cha, D.S. and Chinnan, M.S. (2004). Biopolymer-based antimicrobial packaging: A review. *Critical Reviews in Food Science and Nutrition*, **44**(4), 223–237.

Champagne, C.P. and Fustier, P. (2007). Microencapsulation for the improved delivery of bioactive compounds into foods. *Current Opinion in Biotechnology*, **18**, 184–190.

Champagne, C.P., Raymond, Y., Tompkins, T.A. (2010). The determination of viable counts in probiotic cultures microencapsulated by spray-coating. *Food Microbiology*, **24**, 1104–1111.

Chandramouli, V., Kailasapathy, K., Peiris, P. and Jones, M. (2004). An improved method of microencapsulation and its evaluation to protect Lactobacillus spp. in simulated gastric conditions. *Journal of Microbiological Methods*, **56**, 27–35.

Chen, C.C., Chueh, J.Y., Tseng, H., Huang, H.M. and Lee, S.Y. (2003). Preparation and Characterization of biodegradable PLA polymeric blends', *Biomaterials*, **24**, 1167.

Chen, L., Remondetto, G.E., Subirade, M. (2006). Food protein-based materials as nutraceutical delivery systems. *Trends in Food Science and Technology*, **17**, 272–283.

Cho, M.J., Unklesbay, N., Hsieh, F.H. and Clarke, A.D. (2004). Hydrophobicity of bitter peptides from soy protein hydrolysates. *Journal of Agricultural and Food Chemistry*, **52**, 5895–5901.

Chronakis, I.S. (2005). Novel nanocomposites and nanoceramics based on polymer nanofibers using electrospinning process – A review. *Journal of Materials Processing Technology*, **167**(2–3), 283–293.

Coma, V. (2008). Bioactive packaging technologies for extended shelf life of meat-based products. *Meat Science*, **78**(1–2), 90–103.

Cutter, C.N. and Summer, S.S. (2002). Application of edible coatings on muscle foods. In: A. Gennadios (ed.) Protein-based films and coatings. (pp 467–484). CRC Press, Boca Raon, FL.

Datta, S., Janes, M.E., Xue, Q.G., Losso, J. and La Peyre, J.F. (2008). Control of Listeria monocytogenes and Salmonella anatum on the surface of smoked salmon coated with calcium alginate coating containing oyster lysozyme and nisin. *Journal of Food Science*, **73**, M67–M71.

de Moura, M.R., Aouada, F.A., Avena-Bustillos, R.J., McHugh, T.H., Krochta, J.M. and Mattoso, L.H.C. (2009). Improved barrier and mechanical properties of novel hydroxypropyl methylcellulose edible films with chitosan/tripolyphosphate nanoparticles. *Journal of Food Engineering*, **92**, 448–453.

de Moura, M.R., Avena-Bustillos, R.J., McHugh, T.H., Krochta, J.M. and Mattoso, L.H.C. (2008). Properties of novel hydroxypropyl methylcellulose films containing chitosan nanoparticles. *Journal of Food Science*, **73**(7), N31–N37.

de Moura, M.R., Avena-Bustillos, R.J., McHugh, T.H., Wood, D.F., Otoni, C.G. and Mattoso, L.H.C. (2011). Miniaturization of cellulose fibers and effect of addition on the mechanical and barrier properties of hydroxypropyl methylcellulose films. *Journal of Food Engineering*, **104**(1), 154–160.

Dogan, N. and McHugh, T.H. (2007). Effects of microcrystalline cellulose on functional properties of hydroxyl propyl methyl cellulose microcomposite films. *Journal of Food Science*, **72**(1), E16–E22.

Doherty, S.B., Auty, M.A., Stanton, C., Ross, R.P., Fitzgerald, G.F. and Brodkorb, A. (2012). Survival of entrapped Lactobacillus rhamnosus GG in whey protein micro-beads during simulated ex vivo gastro-intestinal transit. *International Dairy Journal*, **22**, 31–43.

Dorman, H.J. and Deans, S.G. (2000). Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, **88**,308–316.

Dutta, P.K., Tripathi, S., Mehrotra, G.K. and Dutta, J. (2009). Perspectives for chitosan based antimicrobial films in food applications. *Food Chemistry*, **114**(4), 1173–1182.

Fabra, M.J., Busolo, M., López-Rubio, A. and Lagaron, J.M. (2013a). Potential of Nanotechnology to Constitute Biolayers to Enhance Barrier Properties in Foods and Packaging. *Trends in Food Science and Technology*, **31**(1), 79–87.

Fabra, M.J., Chambin, O., Gay, J.P., Voilley, A. and Debeaufort, F. (2012). Influence of temperature and NaCl on the release in aqueous liquid media of aroma compounds encapsulated in edible films. *Journal of Food Engineering*, **108**(1), 30–36.

Fabra, M.J., Hambleton, A., Talens, P., Debeaufort, F., Chiralt, A. and Voilley, A. (2009). Influence of interactions on water and aroma permeabilities of iota-carrageenan-oleic acid-beeswax films used for flavour encapsulation. *Carbohydrate Polymers*, **76**(2), 325–332.

Fabra, M.J., López-Rubio, A. and Lagaron, J.M. (2013b). High barrier polyhydroxyalcanoate food packaging film by means of nanostructured electrospun interlayers of zein prolamine. *Food Hydrocolloids*, **32**(1), 106–114.

Fabra, M.J., Talens, P. and Chiralt, A. (2010). Properties of sodium caseinate films containing lipids. In: *Food Hydrocolloids: Characteristics, Properties and Structures*, S. Hollingworth (ed.) Nova Science Publishers Inc., Hauppauge, NY, USA (pp. 119–144).

Falguera, V., Quintero, J.P., Jiménez, A., Aldemar-Muñoz, A. and Ibarz, A. (2011). Edible films and coatings: Structures, active functions and trends in their use. *Trends in Food Science and Technology*, **22**(6), 292–303.

Fernandez, A., Torres-Giner, S. and Lagaron, J.M. (2009). Novel route to stabilization of bioactive antioxidants by encapsulation in electrospun fibers of zein prolamine. *Food Hydrocolloid*, **23**, 1427–1432.

Fernandez-Saiz, P., Lagaron, J.M. and Ocio, M.J. (2009). Optimization of the film-forming and storage conditions of chitosan as an antimicrobial agent. *Journal of Agricultural and Food Chemistry*, **57**(8), 3298–3307.

Fernandez-Saiz, P., Soler, C., Lagaron, J.M. and Ocio, M.J. (2010). Effects of chitosan films on the growth of Listeria monocytogenes, Staphylococcus aureus and Salmonella spp. in laboratory media and in fish soup. *International Journal of Food Microbiology*, **137**(2–3), 287–294.

Frankel, E. (2005). *Lipid Oxidation*, 2nd edn. The Oily Press: Bridgwater TA7 OYZ, England.

Friedman, M. Henika, P.R., Levin, C.E., Mandrell, R.E. and Kozukue, N. (2006). Antimicrobial activities of tea catechins and theaflavins and tea extracts against Bacillus cereus. *Journal of Food Protection*, **69**, 354–361.

Friedman, M., Henika, P.R. and Mandrell, R.E. (2003). Antibacterial activities of phenolic benzaldehydes and benzoic acids against Campylobacter jejuni, Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella enterica. *Journal of Food Protection*, **66**, 1811–1821.

Friedman, M., Kim, S.Y., Lee, S.J., Han, P.G., Han, J.S., Lee, K.R. and Kozukue, N. (2005). Distribution of catechins, theaflavins, caffeine, and theobromine in 77 teas consumed in the United States. *Journal of Food Science*, **70**, C550–C559.

Ghanbarzadeh, B. and Oromiehi, A. R. (2009). Thermal and mechanical behavior of laminated protein films. *Journal of Food Engineering*, **90**(4), 517–524.

Gouin, S. (2004). Micro-encapsulation: Industrial appraisal of existing technologies and trends. *Trends in Food Science & Technology*, **15**, 330–347.

Guarda, A., Rubilar, J.F., Miltz, J. and Galotto, M.J. (2011). The antimicrobial activity of microencapsulated thymol and carvacrol. *International Journal of Food Microbiology*, **146**(2), 144–150.

Han, C., Zhao, Y., Leonard, S.W. and Traber, M.G. (2004). Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (Fragaria×ananassa) and raspberries (Rubus ideaus). *Postharvest Biology and Technology*, **33**, 67–78.

Han, J.H. (2000). Antimicrobial food packaging. *Food Technology*, **54**, 56–65.

Handley, D., Ma-Edmonds, M., Hamouz, F., Cuppett, S., Mandigo, R. and Schnepf, M. (1996). Controlling oxidation and warmed-over flavor in precooked pork chops with rosemary oleoresin and edible film. In: F., Shahidi (Ed): *Natural Antioxidants Chemistry, Health Effects and Applications*, pp, 311–318. AOCS Press, Champaign, Illinois.

Hargens-Madsen, M., Schnepf, M., Hamouz, F., Weller, C. and Roy, S. (1995). Use of edible films and tocopherol in the control of warmed over flavor. *Journal of the Academy of Nutrition and Dietetics*, **95**, A41.

Haugaard, V.K, Udsen, A.M., Mortensen, G., Hoegh, L., Petersen, K. and Monahan, F. (2001). Food biopackaging. In: C.J. Weber (eds). *Biobased Packaging Materials for the Food Industry – Status and Perspectives*. A report from the EU concerted action project: Production and application of biobased packaging materials for the food industry (Food Biopack), funded by DG12 under the contract PL98 4046. http://www.biomatnet.org/publications/f4046fin.pdf.

Huang., C.H. and Weng, Y.M. (1998). Inhibition of lipid oxidation in fish muscle by antioxidant incorporated polyethylene film. *Journal of Food Processing and Preservation*, **22**(3), 199–209.

Huang, Z.M., Zhang, Y.Z., Kotaki, M. and Ramakrishnan, S. (2003). A review on polymer nanofibers by electrospinning and their applications in nanocomposites. *Composites Science and Technology*, **63**(15), 2223–2253.

Jacobsen, S. and Fritz, H. G. (1996). Filling of Poly(Lactic Acid) with native starch, *Polymer Engineering Science*, **36**, 2799.

Jacobsen, S., Degée, P.H., Fritz, H.G., Dubois, P.H. and Jérome, R. (1999). Polylactide (PLA)A new way of production. *Polymer Engineering Science*, **39**, 1311.

Javeed Akhtar, M., Jacquot, M., Arab-Tehrany, E., Gaïani, C., Linder, M. and Desobry, S. (2010). Control of salmon oil photo-oxidation during storage in HPMC packaging film: Influence of film colour. *Food Chemistry*, **120**, 395–401.

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M. (2008). Antioxidative activity and properties of fish skin gelatin films incorporated with BHT and α-tocopherol. *Food Hydrocolloids*, **22**, 449–458.

Kailasapathy, K. and Champagne, C.P. (2011). Trends in the microencapsulation of probiotics for application in dairy products. *AgroFOOD Industry Hi Tech*, **22**(2), 25–27.

Kanmani, P., Kumar, R.S., Yuvaraj, N., Paari, K.A., Pattukumar, V. and Arul, V. (2011). Effect of cryopreservation and microencapsulation of lactic acid bacterium Enterococcus faecium MC13 for long-term storage. *Biochemical Engineering Journal*, **58–59**, 140–147.

Kilincceker, O., Dogan, I.S. and Kucukoner, E. (2009). Effect of edible coatings on the quality of frozen fish fillets. *LWT-Food Science and Technology*, **42**(4), 868–873.

Kim, Y. M., An, D.S., Park, H.J., Park, J.M. and Lee, D.S. (2002). Properties of nisin-incorporated polymer coating as antimicrobial packaging materials. *Packaging Technology and Science*, **15**, 247–254.

Koening, M.F. and Huang, S.J. (1995). Biodegradable blends and composites of polycaprolactone and starch derivatives. *Polymer*, **36**, 1877.

Krasaekoopt, W., Bhandari, B. and Deeth, H. (2003). Evaluation of encapsulation techniques of probiotics for yoghurt. *International Dairy Journal*, **13**, 3–13.

LaCoste, A., Schaich, K.M., Zumbrunnen, D. and Yam, K.L. (2005). Advancing controlled release packaging through smart blending. *Packaging Technology and Science*, **18**, 77–87.

Lagaron, J.M. (2011). *Multifunctional and Nanoreinforced Polymers for Food Packaging*. Woodhead Publishing Limited, Cambridge, UK.

Lagaron, J.M. and Powell, A. K. (2000). Polyketones having improved barrier properties. Patent WO 0042089. 2000–07–20.

Lagaron, J.M., Giménez, E., Sánchez-García, M.D., Ocio, M.J. and Fendler, A. (2006). Technical Session. 'The 15th IAPRI World Conference on Packaging' Published: International Association of Packaging Research Institutes Japan Packaging Institute. Japan, Tokyo.

Lagaron, J.M., Giménez, E., Sánchez-García, M.D., Ocio, M.J. and Fendler, A. (2007). *Food Contact Polymers*, Chapter 19. Rapra Technologies, Shawbury, UK, ISBN: 978-1-84735-012-1.

Lagaron, J.M., Ocio, M.J. and López-Rubio, A. (2012). Antimicrobial Polymers. Wiley-WCH, Inc., Publications.

Lakkis, J.M. (2007). *Encapsulation and Controlled Release: Technologies in Food Systems*. Blackwell Publishing, Iowa.

Lalush, I., Bar, H., Zakaria, I., Eichler, S. and Shimoni, E. (2005). Utilization of amylose-lipid complexes as molecular nanocapsules for conjugated linoleic acid. *Biomacromolecules*, **6**, 121–130.

Lee, J.Y., Park, H.J., Lee, C.Y. and Choi, W.Y. (2003). Extending shelf-life of minimally processed apples with edible coatings and antibrowning agents. *Lebensmittel-Wissenschaft und –Technologie*, **36**, 323–329.

Li, Y., de Vries, R., Slaghek, T., Timmermans, J., Stuart, M.A.C. and Norde, W. (2009). Preparation and characterization of oxidized starch polymer microgels for encapsulation and controlled release of functional ingredients. *Biomacromolecules*, **10**, 1931–1938.

Lima, A.M., Cerqueira, M.A., Souza, B.W.S., Santos, Ed.C.M., Teixeira, J.A., Moreira, R.A. and Vicente, A.A. (2010). New edible coatings composed of galactomannans and collagen blends to improve the postharvest quality of fruits. *Journal of Food Engineering*, **97**(1), 101–109.

Lin, W.H., Hwang, C.F., Chen, L.W. and Tsen, H.Y. (2006). Viable counts, characteristic evaluation for commercial lactic acid bacteria products. *Food Microbiology*, **23**, 74–81.

López-Fandiño, R., Otte, J., van Camp, J. (2006). Physiological, chemical and technological aspects of milk-protein derived peptides with antihypertensive and ACE-inhibitory activity. *International Dairy Journal*, **16**, 1277–1293.

López-Rubio and Lagaron, J.M. (2012). Whey protein capsules obtained through electrospraying for the encapsulation of bioactives. *Innovative Food Science and Emerging Technologies*, **13**, 200–206.

López-Rubio, A., Lagaron, J.M., Ankerfors, M., Lindström, T., Nordqvist, D., Mattozzi, A. and Hedenqvist, M.S. (2007). Enhanced film forming and film properties of amylopectin using micro-fibrillated cellulose. *Carbohydrate Polymers*, **68**(4), 718–727.

López-Rubio, A., Sánchez, E., Sanz, Y. and Lagaron, J.M. (2009). Encapsulation of living bifidobacteria in ultrathin PVOH electrospun fibers. *Biomacromolecules*, **10**, 2823–2829.

López-Rubio, A., Sánchez, E., Wilkanowicz, S., Sanz, Y. and Lagaron, J.M. (2012). Electrospinning as a useful technique for the encapsulation of living bifidobacteria in food hydrocolloids. *Food Hydrocolloids*, **28**, 159–167.

Ma-Edmonds, M., Hamouz, F., Cuppett, S., Madigo, R. and Schnepf, M. (1995). Use of rosemary oleoresin and edible film to control warmed-over flavor in precooked beef patties. Abstract No. 50–6 IFT Annual Meeting. Anaheim, CA.

Martin, O., Schwach, E., Avérous, L. and Couturier, Y. (2001). Polymer blends and composites from renewable resources. *Starch*, **53**, 372–380.

Martínez-Sanz, M., Olsson, R.T., López-Rubio, A. and Lagaron, J.M. (2011). Development of electrospun EVOH fibres reinforced with bacterial cellulose nanowhiskers. Part I: Characterization and method optimization. *Cellulose*, **8**(2), 335–347.

Martínez-Sanz, M., Olsson, R.T., López-Rubio, A. and Lagaron, J.M. (2012). Development of bacterial cellulose nanowhiskers reinforced EVOH composites by electrospinning, *Journal of Applied Polymer Science*, **124**(2), 1398–1408.

Matalanis, A., Jones, O.G. and McClements, D.J. (2011). Structured biopolymer-based delivery systems for encapsulation, protection and release of lipophilic compounds. *Food Hydrocolloids*, **25**, 1865–1880.

McClements, D.J., Decker, E.A., Park, Y. and Weiss, J. (2009). Structural design principles for delivery of bioactive components in nutraceuticals and functional foods. *Critical Review in Food Science*, **49**, 577–606.

Mei, Y., Zhao, Y., Yang, J. and Furr, H.C. (2002). Using edible coating to enhance nutritional and sensory qualities of baby carrots. *Journal of Food Science*, **67**, 1964–1968.

Miller, B.J. and Cutter, C.N. (2000). Incorporation of nisin into collagen films retains antimicrobial activity against Listeria monocytogenes and Brochothrix thermosphacta associated with ready-to-eat meat products. *Journal of Association Food Drug Official*, **68**(4), 64–77.

Morales-Aizpurúa, I.C. and Tenuta-Filho, A. (2005). Oxidation of cholesterol in mayonnaise during storage. *Food Chemistry*, **89**(4), 611–615.

Moreno, Y., Collado, M.C., Ferrus, M.A., Cobo, J.M., Hernandez, E. and Hernandez, M. (2006). Viability assessment of lactic acid bacteria in commercial dairy products stored at 4°C using LIVE/DEAD® BacLight™ staining and conventional plate counts. *International Journal of Food Science & Technology*, **41**, 275–280.

Nam, S.H., Choi, S.P., Kang, M.Y., Koh, H.J., Kozukue, N. and Friedman, M. (2006). Antioxidative activities of bran extracts from twenty one pigmented rice cultivars. *Food Chemistry*, **94**, 613–620.

Olabarrieta, I., Gällstedt, M., Ispizua, I., Sarasua, J.-R. and Hedenqvist, M.S. (2006). Properties of aged montmorillonite-wheat gluten composite films. *Journal of Agricultural and Food Chemistry*, **54**(4), 1283–1288.

Ostlund, R.E. (2004). Phytosterols and cholesterol metabolism. *Current Opinion in Lipidology*, **15**, 37–41.

Ozkan, G., Simsek, B. and Kuleasan, H. (2007). Antioxidant activities of Satureja cilicica essential oil in butter and in vitro. *Journal of Food Engineering*, **79**, 1391–1396.

Park, H.J. (1999). Development of advanced edible coatings for fruits. *Trends in Food Science and Technology*, **10**(8), 254–260.

Park, E. S., Kim, M.N. and Yoon, J.S. (2002). Grafting of polycaprolactone onto poly(ethylene-co-vinyl alcohol) and application to polyethylene-based bioerodable blends. *Journal of Polymer Science: Part B Polymer Physics*, **40**, 2561.

Pavlath, A.E. and Orts, W. (2009). Edible Films and Coatings: Why, What, and How? In: Edible films and coatings for food applications. M.E. Embuscado and K.C. Huber (eds). Springer. London (pp. 1–24).

Pereda, M., Amica, G., Rácz, I. and Marcovich, N.E. (2011). Structure and properties of nanocomposite films based on sodium caseinate and nanocellulose fibers. *Journal of Food Engineering*, **103**(1), 76–83.

Pereira de Abreu, D.A., Paseiro Losada, P., Maroto, J. and Cruz, J.M. (2011). Natural antioxidant active packaging film and its effect on lipid damage in frozen blue shark (Prionace glauca). *Innovative Food Science and Emerging Technologies*, **12**, 50–55.

Petersen, K., Nielsen, P.K., Bertelsen, G., Lawther, M., Olsen, M.B., Nilsson, N.H. and Mortensen, G. (1999). Potential of biobased materials for food packaging. *Trends in Food Science and Technology*, **10**, 52–68.

Petersen, K., Nielsen, P.V. and Olsen, M.B. (2001). Physical and mechanical properties of biobased materials. *Starch*, **53**, 8, 356.

Picot, A. and Lacroix, C. (2003). Production of multiphase water insoluble microcapsules for cell microencapsulation using an emulsification/spray-drying technology. *Journal of Food Science*, **68**, 2693–2700.

Plackett, D. and Siro, I. (2011). Polyhydroxyalkanoates (PHAs) for food packaging. In: *Multifunctional and Nanoreinforced Polymers for Food Packaging*. J.M. Lagaron, ed. Cambrigde, UK, Woodhead Publishing Limited, pp.498–526.

Quintavalla, S. and Vicini, L. (2002). Antimicrobial food packaging in meat industry. *Meat Science*, **62**, 373–380.

Reineccius, G.A. (2009). Edible films and coatings for flavor encapsulation. In: M.E. Embuscado and K.C. Huber (eds). *Edible Films and Coatings for Food Applications* (pp 269–294). Springer, New York.

Rhim, J.W., Mohanty, K.A., Singh, S. P. and Ng, P.K.W. (2006). Preparation and properties of biodegradable multilayer films based on soy protein isolate and poly(lactide). *Industrial and Engineering Chemistry Research*, **45**, 3059–3066.

Ribeiro, H.S., Ax, K. and Schubert, H. (2003). Stability of lycopene emulsions in food systems. *Journal of Food Science* **68**, 2730–2734.

Ribeiro, C., Vicente, A.A., Teixeira, J.A. and Miranda, C. (2007). Optimization of edible coating composition to retard strawberry fruit senescence. Postharvest Biology and Technology, 44(1), 63–70.

Rojas-Graü, M.A., Soliva-Fortuny, R and Martin-Belloso, O. (2009). Edible coatings to incorporate active ingredients to fresh-cut fruits: A review. *Trends in Food Science and Technology*, **22**(10), 438–447.

Sánchez-Garcia, M.D., Hilliou, L. and Lagaron, J.M.(2010). Nanobiocomposites of Carrageenan, Zein, and Mica of interest in food packaging and coating applications. *Journal of Agricultural and Food Chemistry*, **58**(11), 6884–6894.

Sánchez-González, L., Cháfer, M., Hernández, M., Chiralt, A. and González-Martínez, C. (2011). Antimicrobial activity of polysaccharide films containing essential oils. *Food Control*, **22**(8), 1302–1310.

Sansone, F., Mencherini, T., Picerno, P., d'Amore, M., Aquino, R.P. and Lauro, M.R. (2011). Maltodextrin/pectin microparticles by spray drying as carrier for nutraceutical extracts. *Journal of Food Engineering*, **105**, 468–476.

Shah, N.P., Ali, J.F. and Ravula, R.R. (2000). Populations of Lactobacillus acidophilus, Bifidobacterium spp., and Lactobacillus casei in commercial fermented milk products. *Bioscience and Microflora*, **19**, 35–39.

Shahidi, F. and Naczk, M. (2004). *Phenolics in Food and Nutraceuticals*. CRC Press, Boca Raton, FL.

Shutava, T.G., Balkundi, S.S. and Lvov, Y.M. (2009). (-)-Epigallocatechin gallate/gelatin layer-by-layer assembled films and microcapsules. *Journal of Colloid and Interface Science*, **330**, 276–283.

Siddaramaiah, J.G. (2012). High performance edible nanocomposite films containing bacterial cellulose nanocrystals. *Carbohydrate Polymers*, **87**(3), 2031–2037.

Sinha, R.S. and Bousmina, M. (2005). Biodegradable polymers and their layered silicate nanocomposites: In greening the 21st century materials world. *Progress in Materials Science*, **50**, 962–1079.

Smith-Palmer, A., Stewart, J. and Fyfe, L. (1998). Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Letters in Food Microbiology*, **26**, 118–122.

Sorrentino, A., Gorrasi, G. and Vittoria, V. (2007). Potential perspectives of bio-nanocomposites for food packaging applications. *Trends Food Science Technology*, **18**, 84–95.

Starling, S. (2009). From an 'Alcimed' study. Probiotics must meet Europe's new health claim laws head on. NutraIngredients www.nutraingedients.com/content/view/print/242071. 6 April 2009.

Suppakul, P., Miltz, J., Sonneveld, K. and Bigger, S.W. (2003). Active packaging technologies with an emphasis on antimicrobial packaging and its applications. *Journal of Food Science*, **68**, 408–420.

Takahashi, M., Inafuku, K., Miyagi, T., Oku, H., Wada, K., Imura, T. and Kitamoto, D. (2007). Efficient preparation of liposomes encapsulating food materials using lecithins by a mechanochemical method. *Journal of Oleo Science*, **56**(1), 35–42.

Tharanathan, R.N. (2003). Biodegradable films and composite coatings: Past, present and future. *Trends in Food Science and Technology*, **14**, 71–78.

Torres-Arreola, W., Soto-Valdez, H., Peralta, E., Cárdenas-López, J.L. and Ezquerra-Brauer, J.M. (2007). Effect of a low-density polyethylene film containing butylated hydroxytoluene on lipid oxidation and protein quality of sierra fish (Scomberomorus sierra) muscle during frozen storage. *Journal of Agricultural and Food Chemistry*, **55**, 6140–6146.

Torres-Giner, S., Gimenez, E. and Lagaron. J.M. (2008). Characterization of the morphology and thermal properties of Zein Prolamine nanostructures obtained by electrospinning. *Food Hydrocolloids*, **22**, 601–614.

Torres-Giner, S., Martínez-Abad, A., Ocio, M.J. and Lagaron, J.M. (2010). Stabilization of a nutraceutical omega-3 fatty acid by encapsulation in ultrathin electrosprayed zein prolamine. *Journal of Food Science*, **75**, N69–N79.

Tsuji, H. and Yamada, T. (2003). Blends of aliphatic polyesters. VIII. Effects of poly(L-lactide-co-εcaprolactone) on enzymatic hydrolysis of poly(L-lactide), poly(εcaprolactone), and their blend films. *Journal of Applied Polymer Science*, **87**, 412.

Ustunol, Z. (2009). Edible films and coating for meat and poultry. In: M.E. Embuscado and K.C. Huber (eds). *Edible Films and Coatings for Food Applications* (pp 245–269). Springer, New York.

Vásconez, M.B., Flores, S.K., Campos, C.A., Alvarado, J. and Gerschenson, L.N. (2009). Antimicrobial activity and physical properties of chitosan-tapioca starch based edible films and coatings. *Food Research International*, **42**, 762–769.

Weber, C.J., Haugaard, V., Festersen, R. and Bertelsen, G. (2002). Production and application of biobased packaging materials for the food industry. *Food Additives and Contaminants*, **19**, 172.

Weese, J.S. (2002). Microbiologic evaluation of commercial probiotics. *Journal of American Veterinary Medical Association*, **220**, 794–797.

Were, L.M., Bruce, B.D., Davidson, P.M. and Weiss, J. (2003). Size, stability and entrapment efficiency of phospholipid nanocapsules containing polypeptide antimicrobials. *Journal of Agricultural Food and Chemistry*, **51**(27), 8073–8079.

Willcox, J.K., Ash, S.L., Catignani, G.L. (2004). Antioxidants and prevention of chronic disease. *Critical Reviews in Food Science*, **44**, 275–295.

Wong, D. W.S., Caminard, W.M. and Pavlath, A.E. (1994). Development of edible coatings for minimally processed fruits and vegetables. In: J.M. Krochta, E.A. Baldwin and M. Nisperos-Carriedo (eds). *Edible Coatings and Films to Improve Food Quality* (pp 65–88). Technomic, Lancaster, PA.

Wu, Y., Weller, C.L., Hamouz, F., Cuppet, S.L. and Schnepf, M. (2002). Development and application of multicomponent edible coatings and films: A review. *Advances in Food and Nutrition Research*, **44**, 347–394.

Yu, H. and Huang, Q. (2010). Enhanced in vitro anti-cancer activity of curcumin encapsulated in hydrophobically modified starch. *Food Chemistry*, **119**, 669–674.

Zhai, M., Zhao, L., Yoshii, F. and Kume, T. (2004). Study on antibacterial starch/chitosan blend film formed under the action of irradiation. *Carbohydrate Polymers*, **57**, 83–88.

Zhong, Y., Song, X. and Li, Y. (2011). Antimicrobial, physical and mechanical properties of kudzu starch–chitosan composite films as a function of acid solvent types. *Carbohydrate Polymers*, **84**, 335–342.

16

Smart polymers for optical data storage

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Abstract: Smart polymers based on photoresponsive azobenzene moieties have been extensively explored as potential materials for high capacity optical storage. This chapter reviews different strategies to incorporate azobenzenes into polymeric structures as well as their photoresponse properties. The potential of this type of materials as volume holographic media is summarized with special emphasis on block copolymers and blends. Although side-chain azopolymers has been the most investigated, new macromolecular architectures have been recently proposed and will be briefly presented.

Key words: azopolymers, block copolymers, photoresponsive, holographic storage, polymeric blends.

16.1 Introduction

The expansion of Information and Communication Technology (ICT) has had an enormous impact on the socio-economic progress of humankind. Modern digital society requires continuous development of the means to generate, distribute and store increasing amounts of information in a fast and safe fashion. This progress has been possible in part due to advances in different areas of material science and technology. For example, the continuous development of photolithographic techniques and polymeric photoresists has kept pace with Moore's law, doubling the number of transistors per unit area on an integrated circuit every two years. Another key element in this information revolution has been the development of information storage technologies. Nowadays, there are several types of information storage systems, with the most common ones being magnetic hard drives, magnetic tapes, optical discs and solid-state drives (flash memory). In all cases, information is stored as a change in a physical property of a material or device. Flash memory stores information as electronic charge in a solid-state memory cell, while changes in magnetic or optical properties are used to store data in magnetic and optical discs. All of these technologies coexist, each

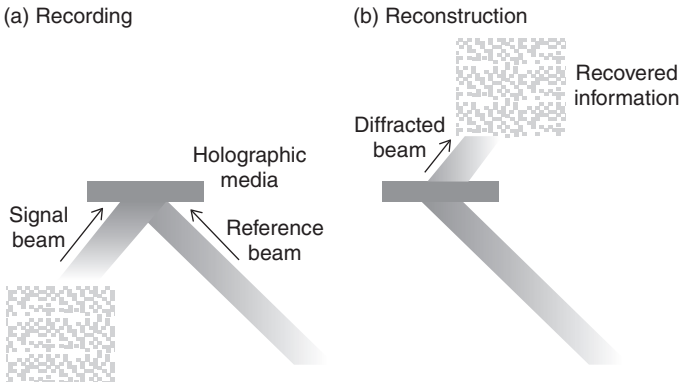
having pros and cons with respect to access speed, durability, capacity and price, and so the needs of each application dictate the choice of equipment.

In conventional optical discs, binary data is usually encoded on the surface as local changes in optical properties following a spiral track. In read only memory (ROM) type discs, bits are encoded as pits and lands in a reflective surface (e.g., aluminum). Injection molding of the pits and lands in polycarbonate followed by metallization of the pattern is the most common method for the production of ROM optical discs. In writable discs (CD-R and CD-RW), information can be registered by locally modifying the optical properties of a photosensitive material film by using a focused laser. Either organic dye layers that generate pits upon irradiation, or inorganic films that experience photoinduced transitions between amorphous and crystalline phases with different reflectivity, are used in these writable systems. In both ROM and writable systems, information can be retrieved by illuminating the data path with a laser and analyzing the reflected light with an optical detection system. The information has to be stable over time and the reading should not affect the recorded information. Since a focused laser is used for reading, data is accessed sequentially (bit by bit), which limits the speed of data access.

Since the introduction of compact discs (CDs), strategies to increase disc capacity using multilayers or double sided discs have been employed. The increase in optical storage capacity in successive generations, from compact discs to digital versatile discs (DVDs) to blu-ray discs (BDs), has been achieved using lasers with shorter wavelengths (780 nm in CD, 650 nm in DVD and 405 nm in BD). The size of the focused beam used to record or read is proportional to its wavelength and therefore this wavelength reduction is an effective way to increase storage capacity. Attempts are now being made to implement a fourth generation of optical discs using blue-violet lasers; however, there are additional difficulties with the three first generations due to the absorbance of materials and optics in this spectral region (Watabe, 2011).

The storage capacity of conventional two-dimensional optical discs is limited by light diffraction. Research has been performed to investigate the use of confined optical fields using near-field optical recording. The optical field that records or reads the information in the storage material is confined by a nanometer sized object, such as a metal-coated, near-field optical fiber, which imposes a nanometer scale lateral resolution and so significantly increases storage capacity. Despite this remarkable advance, transfer speeds suffer from the same limitations as the other 2D technologies, and the added complexity of the optical head disc could be considered a disadvantage.

Holographic storage is an alternative optical technology with significantly improved storage capacity and information transfer rates. Using a completely different approach, holography makes use of the whole volume



16.1 Hologram (a) recording and (b) reconstruction.

of the recording material, boosting the storage capacity of a single disc. In addition, it is possible to record or read a complete page of information (containing thousands of bits) in one single event, enormously increasing the transfer rate compared to conventional, bit by bit reading, optical discs.

Holographic data storage makes use of two coherent light beams that interfere in the recording medium, as schematically depicted in Fig. 16.1. Information is recorded using a signal beam, which carries digital data as a spatially modulated wavefront, and a reference beam with no information encoded. These two beams are made to overlap at a certain angle in the photosensitive storage material. The three dimensional interference pattern generated in the overlapping region is captured by the holographic material as a change in a physical property (e.g., absorption, refractive index, optical anisotropy, etc.). To read the registered information, the reference beam illuminates the recorded hologram. Detection of the spatially modulated diffracted beam allows the recovery of previously registered information.

For sufficiently thick recording media, usually in the order of hundreds to a thousand micrometers, the hologram recorded is classified as a thick or volume hologram in comparison to thin holograms. Volume holograms offer interesting features for information storage applications. Illumination with the reference beam of a thick hologram results in diffraction in one single order (apart from order 0) at the Bragg angle. Slight detuning of the incident angle results in loss of the diffraction condition. This high angular selectivity of volume holograms allows multiplexing several holograms in the same volume of material, with each being independently recovered, boosting the storage capacity of the system.

The idea for holographic data storage arose in the early 1960s, but its commercial introduction has been a slow process. Despite the advances in systems design and materials, mainstream storage technologies have evolved

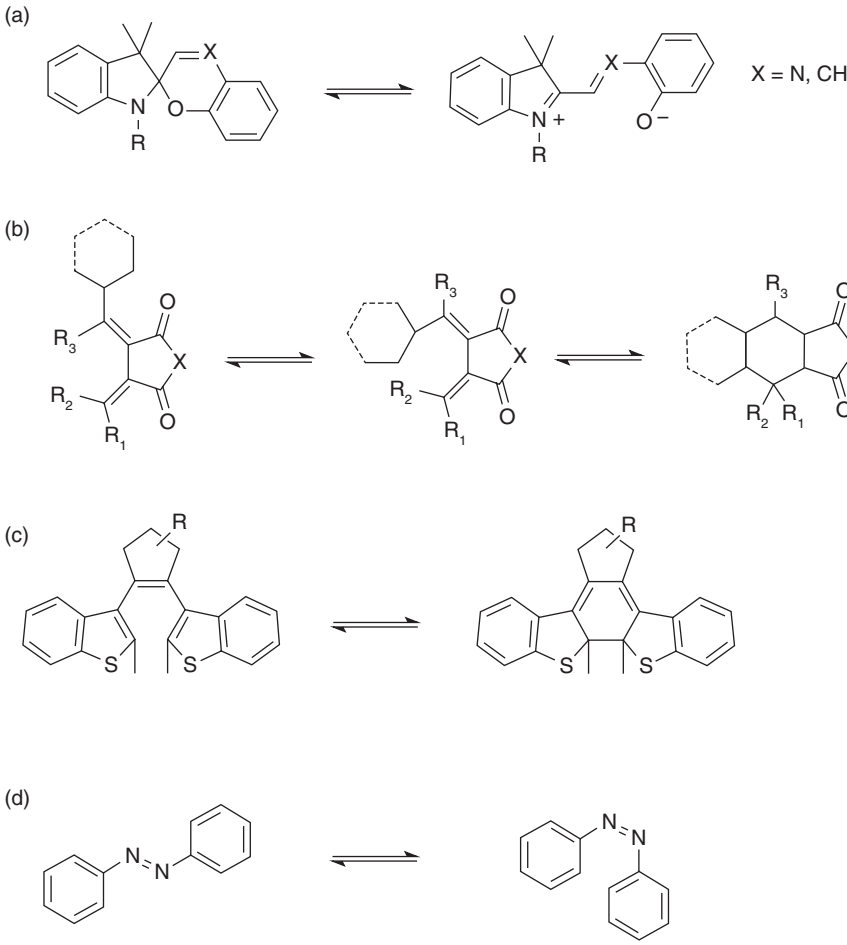
even faster and they have narrowed the market for holographic storage. Materials development is a major issue in developing holographic optical storage technology due to the demanding storage media requirements, the most important being:

- good optical quality with low scattering losses;
- good sensitivity, which allows rapid recording of holograms and is energy-efficient;
- a large dynamic range, with the medium able to multiplex several holograms in the same volume;
- low optical absorption, so that the whole volume of the recording media can be sensitized; and
- stability of the recorded holograms.

If the recording medium needs to be rewritable, it should be possible to erase recorded information, preferentially in a local fashion, for example using light.

The search for volume holographic materials that fulfill all the previously described properties has been an active topic of research for several decades. However, despite these intensive efforts, further research is still needed (Bruder *et al.,* 2011). Inorganic materials showing photorefractivity (e.g., $LiNiO_3$, $KNiO_3$) have been explored as suitable reversible volume holographic materials (Chen *et al*., 1968). Polymeric systems have also been investigated, especially those based on photopolymers in which light triggers a polymerization reaction leading to a change in the refractive index of the material (Colburn and Haines 1971). Smart polymers containing photochromic units, which experience reversible photoinduced transformations between two states with different absorption spectra, such as those represented in Fig. 16.2, have also been studied as rewritable holographic materials.

For example, light-induced pericyclic reactions have proven suitable for optical data storage (Shibaev *et al*., 2003).Upon UV irradiation, spiropyrans and spiro-[1,4]-oxazines are converted from the colorless or slightly colored spirocyclic closed-form to the deeply colored merocyanine open-form by an electrocyclic ring opening, which is the basis of its application for optical memories (Berkovic *et al.,* 2000). The merocyanine form possesses a zwitterionic structure that has a high tendency to aggregation. Fulgides and diarylethylenes can also be reversibly converted between an open- and a closed-form by a photoinduced 6π-electrocyclization and have also been investigated for this application (Lenoble and Becker, 1986). However, the versatility of the azobenzene moiety, where chemical modifications are easily incorporated, made this unit an excellent chromophore for incorporation into polymeric structures. In fact, azobenzene derivatives are widely studied



16.2 Photochromic moieties: (a) spiropyrans and spiro[1,4]oxazines, (b) fulgides, (c) diarylethylenes and (d) azobenzenes.

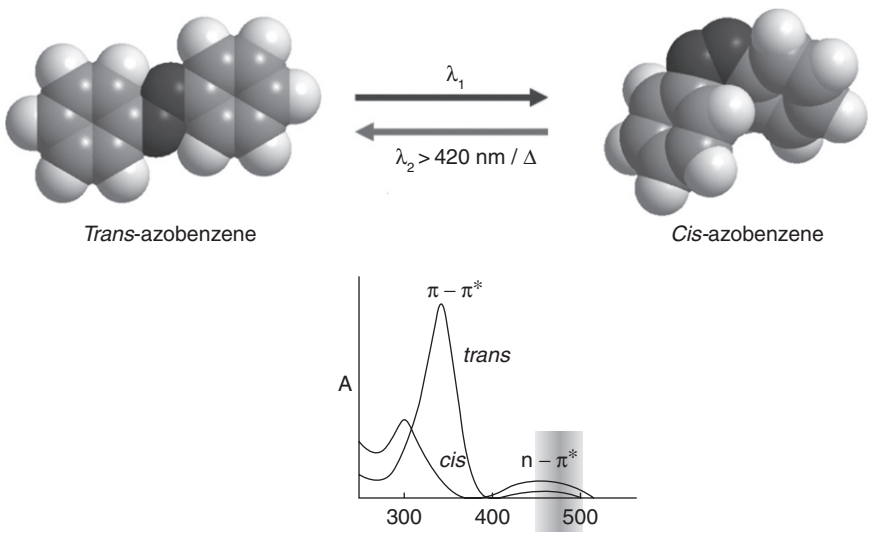
photochromic moieties in the design of smart polymers for optical applications and, in particular, as candidates for rewritable storage media.

This chapter focuses on this particular family of photochromic systems. As presented elsewhere in this book, azobenzene-containing materials experience reorganizations at different length scales in response to light of the appropriate wavelength. The photochemistry of azobenzene molecules will be described. The main types of azobenzene polymeric architectures will be presented as well as associated synthetic methods. A brief overview of the photoresponse of azobenzene polymers when exposed to linearly polarized light in their absorption bands will be presented. Special emphasis will be

placed on research into azobenzene materials for volume holography. New azobenzene polymeric architectures that have recently been explored and their photoresponse properties will also be summarized here.

16.2 Photoinduced molecular motions of azobenzene chromophores

Azobenzene molecules exist as two different isomers, *trans* and *cis*, as shown in Fig. 16.3. The *trans* isomer has a rod-like molecular shape and is thermodynamically more stable than the *cis* one, which has a bent molecular shape. Azobenzene can undergo reversible, photoinduced isomerizations between these two states when irradiated in their absorption bands. Absorption of the azo isomers is usually characterized by π-π* and *n*-π* transitions whose positions strongly depend on the azobenzene substituents (Bandara and Burdette, 2012). When the optical absorption of the *trans* and *cis* isomers overlaps, irradiation at this overlapping wavelength range usually results in efficient *trans-cis-trans* isomerization cycles during which molecular reorientation can take place. The transition dipole moment of the *trans* isomer is parallel to the long molecular axis and, due to the selection rules of the absorption process, irradiation with linearly polarized light usually leads



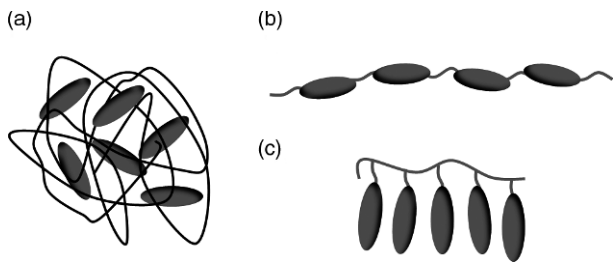
16.3 Reversible *trans-cis-trans* isomerization of azobenzene derivatives and representative absorption spectra of *trans* and *cis* isomers (overlapped region of n-**π*** transition usually used for reversible isomerization cycles).

to a population of molecules lying with their long axis perpendicular to the polarization direction of the incident light. This anisotropic molecular orientation, together with the anisotropy of the molecular polarizability, leads to a large macroscopic birefringence in the irradiated areas. This photoinduced change of the optical anisotropy has led to the study of these materials as thin layers for bit-wise 2D recording or holographic media (Hagen and Bieringer, 2001).

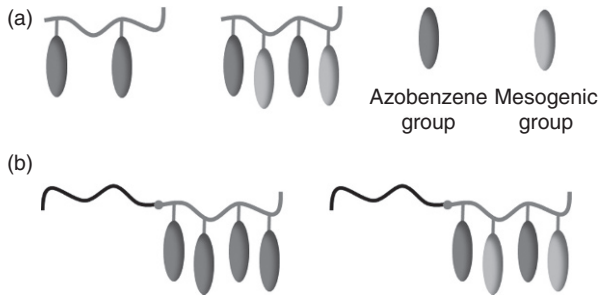
16.3 Macromolecular architectures in azopolymers

During the last two decades, a wide variety of azobenzene-containing polymers has been explored with the aim of obtaining suitable materials for optical storage applications with high sensitivity and stable photoinduced birefringence. Incorporating the chromophore into the polymeric structure is an important issue in the molecular design of these materials. Azobenzenes have conventionally been incorporated into polymeric materials according to the systems schematically represented in Fig. 16.4: (a) host–guest, where azobenzenes are physically dispersed into a polymeric matrix; (b) linear main-chain polymers, where azobenzene moieties are part of the polymeric backbone; and (c) linear side-chain polymers with the azobenzene moieties as side pendant groups covalently linked to the polymeric chain. Azobenzene side-chain polymers are the foremost architecture of choice in exploring the potential of these materials as optical data storage media. In these materials, the photoresponsive units are usually attached to the polymeric chain by means of a flexible spacer that facilitates photoorientation, according to the macromolecular design first introduced by Ringsdorf and Schmidt (1984) in the field of liquid crystalline polymers.

Holographic storage requires additional material properties. Due to the large extinction coefficient of the chromophore at recording wavelengths, films with high azobenzene contents are highly absorbent. As a result, the recording light cannot penetrate more than a few micrometers through the



16.4 Azobenzene incorporation into polymeric materials: (a) guest–host systems (b) linear main-chain and (c) linear side-chain polymers.



16.5 Schematic representation of the principal type of copolymers based on azobenzene: (a) statistical copolymers with non-absorbing monomers (non-mesogenic or mesogenic) and (b) block copolymers.

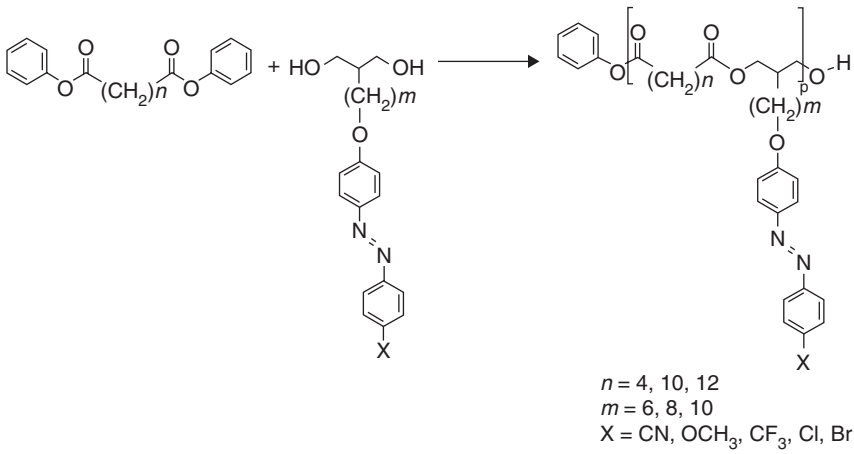
film, precluding the use of these materials in volume holography. To circumvent this problem, the azobenzene content is diluted using strategies such as statistical copolymerization with monomers (either non-mesogenic or mesogenic) that do not absorb the recording light (Fig. 16.5a), or the preparation of block copolymers (BCs) (Fig. 16.5b) with an azoresponsive block and a non-absorbing one. Both approaches aim to dilute the azobenzenes but the second strategy ensures proximity of the chromophoric moieties. This last factor has been discovered to have a large influence on the final photoresponse of these materials.

16.4 Synthetic strategies to azopolymers for optical data storage

Macromolecules with well-defined structures carrying azobenzene units can be obtained by two synthetic strategies, which consist either of polymerizing the appropriate monomers or appending the functional unit onto an existing polymer by post-polymerization reactions. The first method provides precise structural control over the final polymer. However, preparing polymers with high, controlled levels of functional groups by polymerization of azomonomers is limited by the availability and reactivity of such monomers. An attractive and versatile alternative is the post-modification of a polymeric precursor. In this case, an incomplete functionalization of the polymeric skeleton is the major disadvantage.

16.4.1 Synthetic approaches to azobenzene-containing homopolymers and statistical copolymers

In order to tailor the thermal properties and photoresponse of azopolymers, structural modifications of polymers and chromophore moieties are needed.



16.6 Azobenzene polyesters synthesized by step polymerization (melt transesterification).

These modifications include variations on the length of the spacer which connects the azobenzene and the polymeric chain in side-chain azopolymers, or the electronic nature of substituents at the azobenzene moiety (Ruhmann, 1997). This requires the synthesis of appropriate monomers. Conventional techniques can be used for the polymerization of azobenzene monomers. For instance, step polymerization has been used by Hvilsted and co-workers to synthesize different series of liquid crystalline polyesters with potential applications in reversible optical data storage. These polyesters have a side-chain architecture, which synthesis is represented in Fig. 16.6 (Hvilsted *et al.,* 1995). Being a modular synthetic approach, the influence of different structural parameters on the photoinduced optical properties can be evaluated in relatively simple manner.

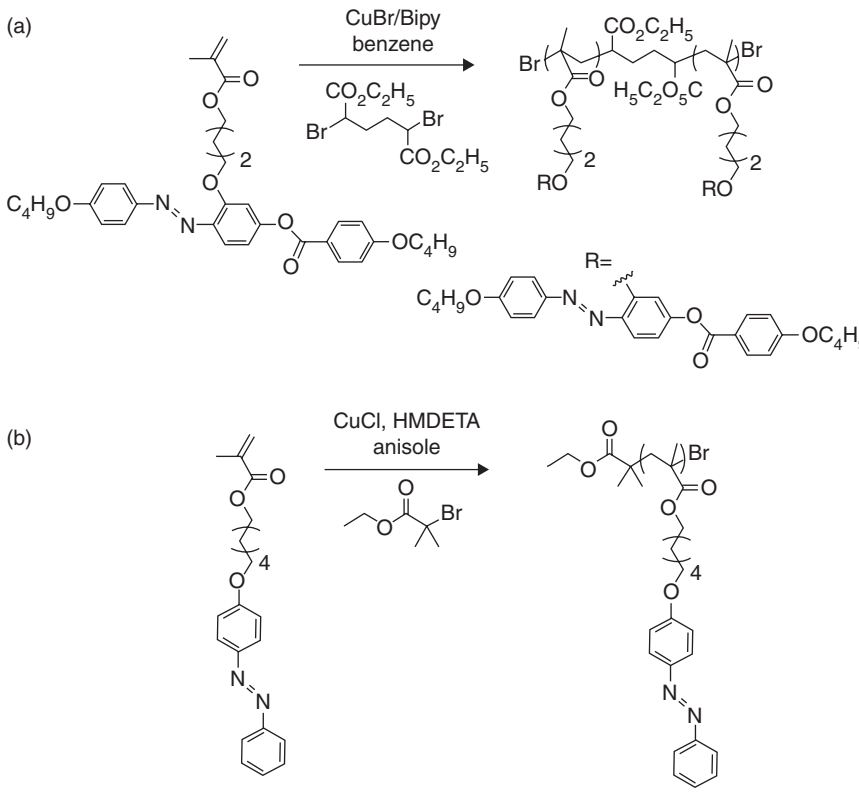
The vast majority of azopolymers developed for optical storage are polyacrylates and polymethacrylates, which are generally prepared by free radical chain polymerization in solution using conventional experimental conditions. For example, azobisisobutyronitrile (AIBN) is used as a thermal initiator in dry organic solvents such as *N,N*-dimethylformamide (DMF), tetrahydrofuran (THF) or dioxane as the most common. Occasionally, the polymerization process of azobenzene (meth)acrylates can be limited by the radical transfer reaction promoted by the azo group, which seems to be associated with the formation of hydrazyl radicals (Nuyken and Weidner, 1986; Hallensleben and Weichart,1989).

The preparation of statistical copolymers based on azobenzene monomers and comonomers of different natures is a simple synthetic pathway for tailoring material properties. These properties can be controlled by varying

the structure of the comonomers and their molar ratio (Ishiguro *et al.*, 2007; Sahishoji *et al.,* 2007). Copolymerization can be carried out with different purposes, such as dilution of the azobenzene content in the final material by copolymerization with non-absorbing monomers (at the writing and reading wavelength), or the incorporation of additional functional moieties, or to improve thermal and optical properties. The copolymerization process does not create any additional difficulties, because the monomers are fed together in the polymerization vial, and polymerization is conducted under the same experimental conditions as for homopolymers. Provided the comonomers have similar reactivity, a good agreement between the feed comonomer ratio and the copolymer composition is usually reached (Angiolini *et al.,* 2007; Czapla *et al.*, 1993), rationalizing slight deviations from feed composition with the stiffness and steric hindrance of the comonomers or to the presence of groups (e.g., nitro group), which act as retarders in free radical polymerizations (Angiolini *et al.*, 2007). In this last case, larger amounts of initiator and longer polymerization times are required to obtain only moderate polymerizations yields (Cojocariu and Rochon, 2005).

Progress in recent decades in controlled radical polymerization (CRP) techniques, including atom transfer radical polymerization (ATRP), (Jin *et al.*, 2004; Li *et al.*, 2002) or reversible addition fragmentation chain transfer (RAFT) polymerization (Gao *et al.,* 2007; Xu *et al.*, 2008; Zhang *et al.*, 2007b), has enabled better control over molecular weight and molecular weight distribution, amongst other features of the polymer architecture. The ATRP technique has been applied to the preparation of azopolymers with narrow molecular weight distributions and controllable molecular weights. Keller *et al.* described the first polymer obtained by ATRP from an azobenzene methacrylate (Li *et al.*, 2002) and since then, the technique has been extensively described for the preparation of azopolymers for different purposes. Alkyl bromides such as 2-bromoisobutyrate derivatives as the initiator radical, and Cu(I) metal salts (CuBr or CuCl) in combination with nitrogen ligands such as *N,N,N′,N″,N‴,N⁗*-hexamethyltriethylenetetramine (HMTETA), *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDETA) or bipyridine ligands in DMF or anisole are most commonly used for the ATRP polymerization of (meth)acrylate azomonomers (Fig. 16.7).

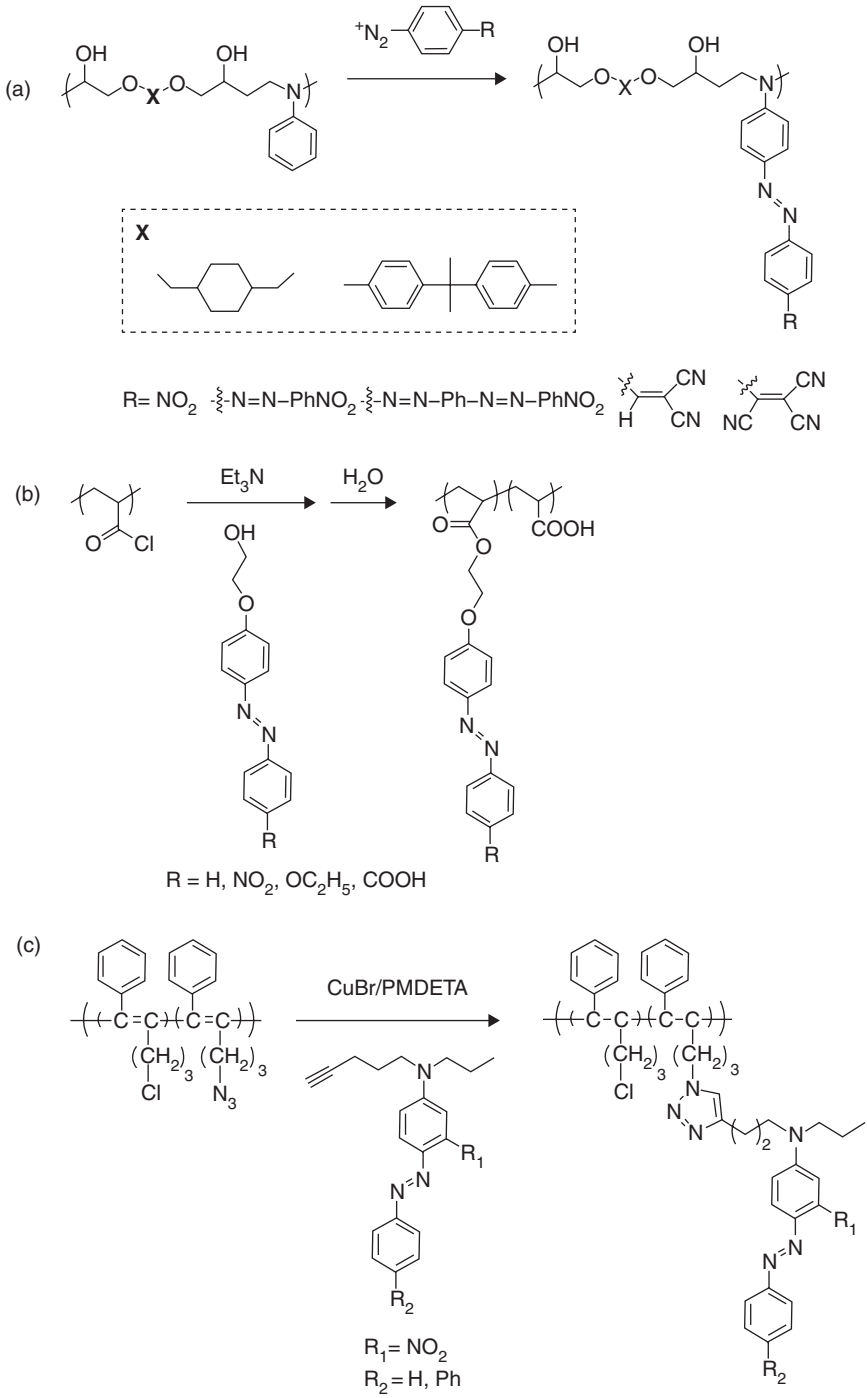
An alternative to the polymerization of appropriate azomonomers is the postfunctionalization of a polymeric skeleton that has reactive groups in the repeating unit. The main advantage of this method is that it prevents retardation of radical polymerization caused by the azo group (Cojocariu and Rochon, 2005). However, highly effective and reliable reactions are required to obtain polymers with well-defined and reproducible macromolecular structures.



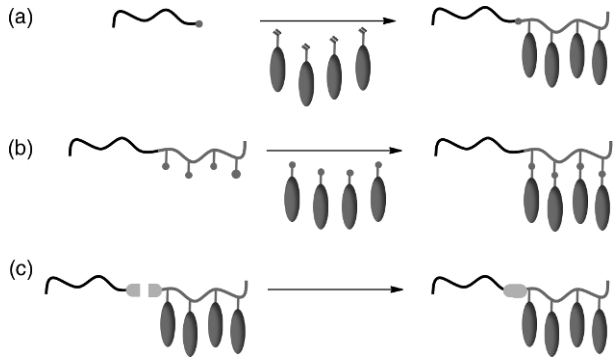
16.7 (a, b) Azobenzene homopolymers prepared by ATRP.

High yield reactions, such as azocoupling and esterification, have been used in the polymers collected in Fig. 16.8a and 16.8b (Kang *et al.*, 2006; Wang *et al.*, 1997). Recently, click chemistry reactions have opened up new possibilities in materials and polymer science and, in particular, in the effective side chain functionalization of macromolecules. The Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) is the most typical reaction employed for this purpose, as in the example shown in Fig. 16.8c (Zeng *et al.*, 2007).

Another representative example concerns azotolane repeating units, which have a positive influence on photoinduced birefringence. However, the synthesis of azotolane monomers requires long and laborious synthetic effort. A simple method for obtaining copolymers with these units was presented by Yu and co-workers, and consists of post Sonogashira cross-coupling to create an azotolane on an azopoly(methacrylate), previously obtained by ATRP, controlling the degree of functionalization under mild conditions with high yields (Yu *et al.,* 2009).



16.8 Azobenzene polymers prepared by postfunctionalization reactions: (a) azocoupling, (b) Schotten-Baumann reaction and (c) CuAAC coupling.



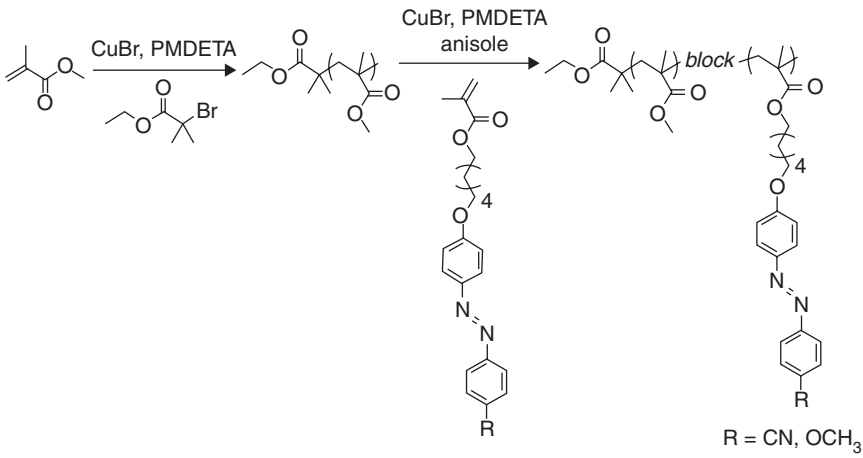
16.9 Synthetic approaches to azobenzene BCs: (a) sequential polymerization: synthesis of macroinitiator and subsequent direct polymerization of azomonomers; (b) postfunctionalization; (c) coupling of preformed blocks.

16.4.2 Synthetic approaches to azobenzene-containing block copolymers

Block copolymers are an interesting alternative for diluting chromophores in order to prepare thick polymeric films for volume holography. Synthesis of BCs containing photoresponsive units can be accomplished using the conventional synthetic strategies shown in Fig. 16.9. They can be synthesized by sequential polymerization of the corresponding monomers, including a monomer containing the functional unit (Fig. 16.9a). Alternatively, BCs can be synthesized by postfunctionalization of a conventional block copolymer (having reactive groups in the repeating units of one of the blocks) by means of an effective polymer-analogous reaction (Fig. 16.9b). The last alternative is to synthesize the corresponding homopolymers and then coupling the preformed blocks (Fig. 16.9c).

Anionic and cationic polymerization are the conventional techniques of preparing BCs, but these processes are effective only if conditions are stringently controlled, and give limited results for vinylic azomonomers (Altomare *et al.*, 1997; Yoshida *et al.,* 2005). CRPs have facilitated access to well-defined BCs and the increasing attention focused on azobenzene BCs.

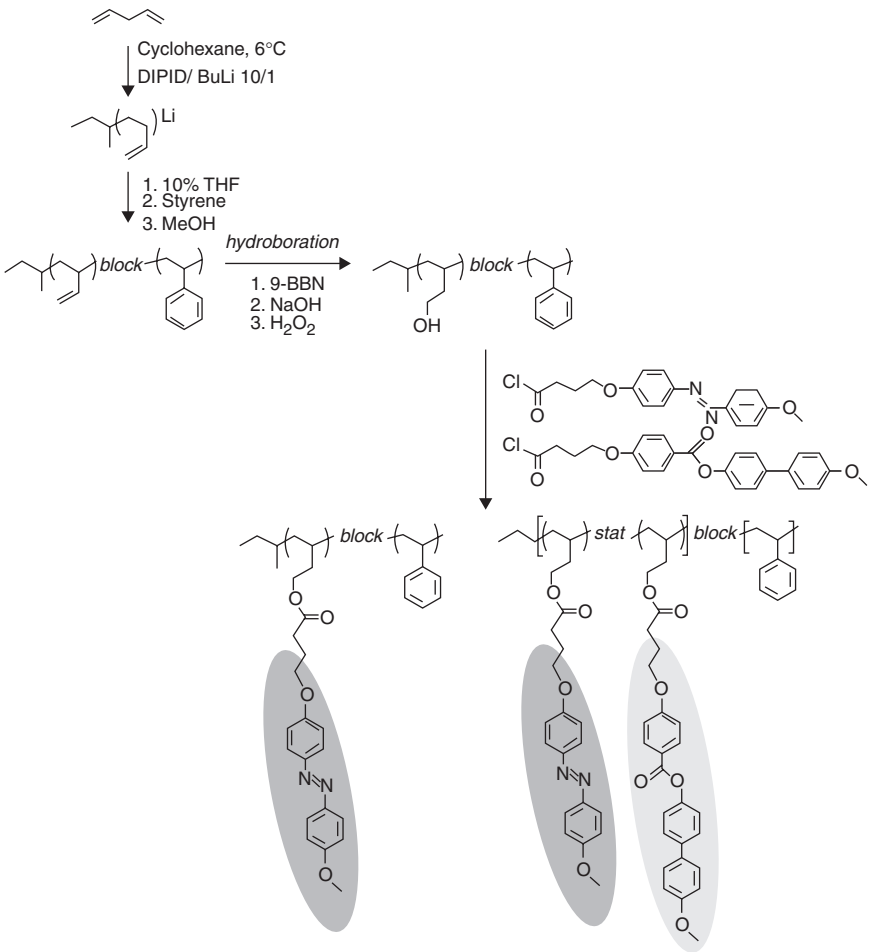
Of particular interest is ATRP, which has been used to prepare azobenzene BCs with poly(methylmethacrylate) (PMMA) (Yu *et al.,* 2008) PS, (Chen *et al.* 2011; Cui *et al.*, 2003), poly(n-butyl acrylate), (Deng *et al.*, 2008) or poly(ethylene) glycol (PEG) blocks (Kadota *et al.*, 2005; Tang *et al.*, 2007) amongst others (see a representative example in Fig. 16.10). The ATRP was successfully used by Matyjaszewski and co-workers to prepare well-controlled 4-cyanoazobenzenemethacrylate homopolymers and BCs



16.10 Synthesis of azobenzene BCs by sequential ATRP (Forcén *et al.,* 2007b).

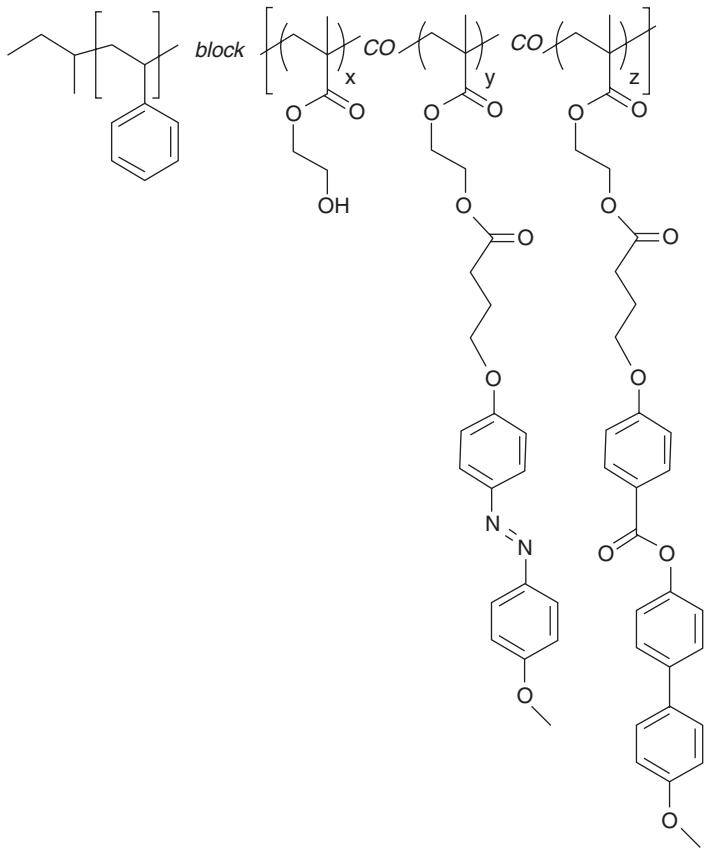
with n-butyl acrylate segments under various experimental conditions. To prepare the BCs, poly(n-butyl acrylate) was grown first and then used to produce the azoblock (Chen *et al.,* 2011). The same strategy has been used by Ikeda and co-workers, where a bromo-terminated PMMA was used as a macroinitiator to obtain diblock copolymers, diblock random copolymers incorporating 4-cyanobiphenyl moieties in the photoaddressable block (Yu *et al.,* 2008), or triblock copolymers where the azoblock was the terminal block (Yu *et al*., 2007). In general, the non-azoic monomer is first polymerized and used as a macroinitiator for the subsequent polymerization of an azobenzene monomer ('non-absorbing block first' route). The reverse approach ('azo-block first' route), which involves synthesis of an azomacroinitiator to carry out the ATRP, has been unsuccessfully attempted by some authors (Forcén *et al*., 2007a). Compared to ATRP, RAFT polymerization has been employed less frequently in this field, although successful RAFT copolymerization of methyl methacrylate using a macro-RAFT agent containing azobenzene moieties ('azo-block first') has been described (Zhang *et al.,* 2007b).

Photochromic units can also be introduced to a BC architecture by means of a polymer-analogous reaction. For this purpose, the previously synthesized BC should contain one block with reactive groups. The main advantage of this strategy is better control over the molecular weight and composition of the initial BC (Adams and Gronski, 1989). Using this approach, Schmidt and collaborators have described a very interesting family of BCs and characterized its performance as holographic recording materials. They developed different azo BCs in three steps (Fig. 16.11). First,



16.11 Synthetic strategy developed by Schmidt and collaborators to approach azo BCs for holographic recording.

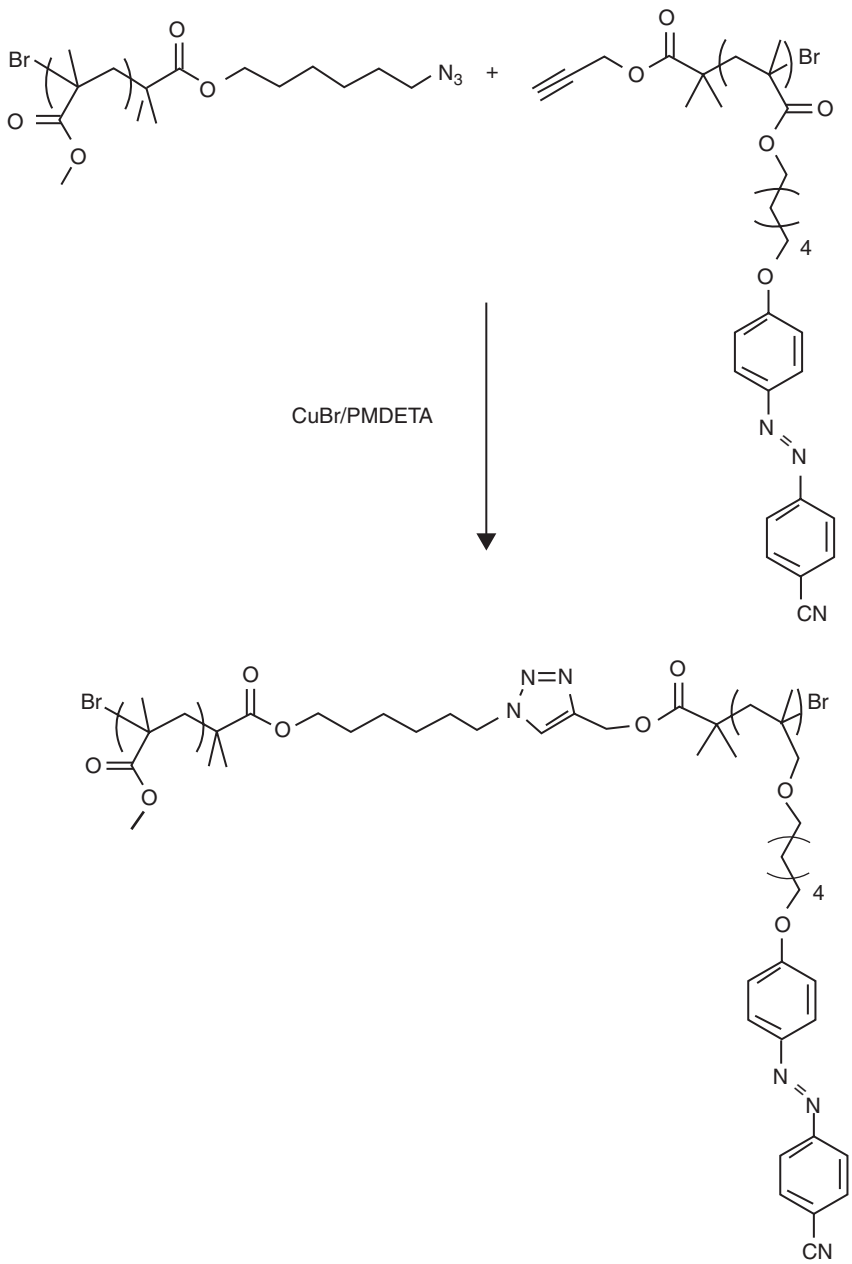
poly(1,2-butadiene)-*block*-polystyrene was synthesized by sequential living anionic polymerization. In the second step, the pendant double bonds were hydroborated to yield functional hydroxyl groups in a highly efficient manner. Finally the hydroxyl groups were functionalized by reaction with acid chloride derivatives (esterification analogous reaction) to covalently attach the azo moieties (Frenz *et al.*, 2004) or a mixture of azo and mesogenic moieties (Häckel *et al.*, 2005a) to the polymeric chain. Similarly, copolymers based on PMMA-*block*-poly(hydroxyethyl methacrylate) (Breiner *et al.*, 2007) or PS-*block*-poly(hydroxyethyl methacrylate) (Häckel *et al.,* 2007) were prepared, in which the functional moieties were again covalently linked



16.12 BCs described by Schmidt and collaborators for the preparation of blends for holographic storage (Häckel *et al.,* 2007).

to the hydroxyl groups by an esterification reaction to yield the azobenzene BCs, as shown in Fig. 16.12. Azobenzene postfunctionalization has also been approached using the azoic coupling reaction (Wang *et al.*, 2008, 2009).

An alternative strategy involves synthesizing and then coupling the corresponding blocks. This is a versatile synthetic approach that allows the preparation of a library of BCs by combining blocks of selected molecular weights or chemical composition. However, this approach relies on the availability of highly efficient and selective coupling reactions under mild conditions, as well as the introduction of reactive and well-defined terminal groups. A combination of CRP, which allows the synthesis of polymers with reactive ending groups, and click chemistry is the best option for this approach as demonstrated in the example shown in Fig. 16.13. PMMA was synthesized by using an ATRP-initiator containing an azide group, and an azopolymer



16.13 Synthetic approach to BCs by click chemistry (Berges *et al.,* 2012a).

block was also prepared by ATRP but using an ATRP-initiator containing an alkyne group. Both blocks were finally coupled by CuAAC click chemistry (Berges *et al.* 2012a).

A similar synthetic protocol was employed for the preparation of BCs containing azopolyesters and PMMA blocks, in this case combining step polymerization, CRP and click chemistry. The azopolyester was first synthesized by transesterification polymerization and subsequently functionalized at the end positions. The PMMA block was then prepared by ATRP using an alkyne-containing initiator and finally the preformed blocks were coupled by CuAAC reaction (Berges *et al.*, 2012b).

16.5 Photoinduced response of azobenzene polymers

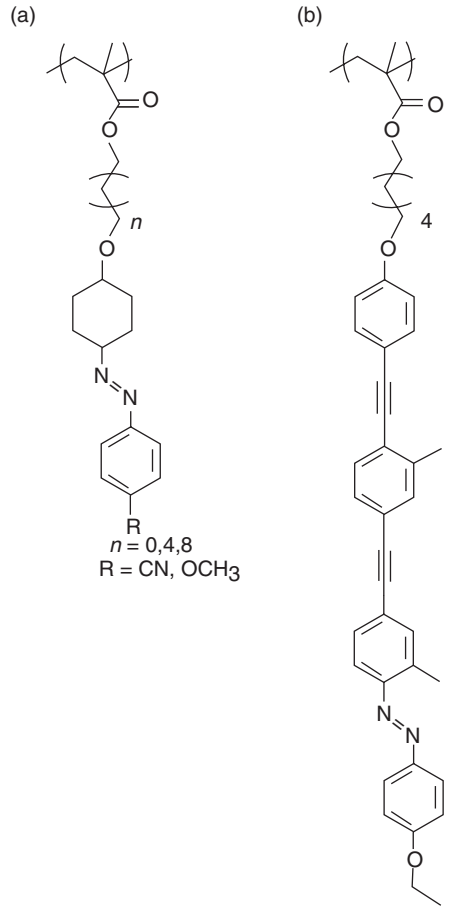
The photoinduced properties of azobenzene systems are strongly related to their polymeric architecture. The way in which azobenzene units are bound to the polymeric chain and the nature, either amorphous or liquid crystalline, of the final material strongly affects the magnitude and stability of the photoinduced response (Natansohn and Rochon, 2002).

Early reports on the photoinduced anisotropic properties in azopolymers described the behavior of guest–host systems composed of azobenzene chromophores dispersed in an amorphous polymeric matrix. Using a dispersion of methyl orange in polyvinyl alcohol, Nikolova and co-workers demonstrated photoinduction of birefringence and recording of polarization holograms (Todorov *et al.*, 1984). These studies served to revitalize the field of photoinduced phenomena in azobenzene materials and allowed experimental demonstration of the peculiar properties of polarization holograms, in particular their selectivity with respect to polarization of the reconstructing beam (Nikolova and Todorov, 1984). This guest–host approach is the easiest strategy for obtaining azo-based materials. It provides the necessary processability and mechanical characteristics of the polymeric host material while the optical properties can be, to some extent, modulated by adjusting the composition of the mixture. For example, the stability of the photoinduced anisotropy can be improved using high T_g host polymers (Natansohn *et al.*, 1995) while the optical absorption can be modulated by changing the chromophore concentration. This latter possibility is especially important in volume holography in which thick films in the order of hundreds of micrometers are needed. Several studies of holographic recording were performed on thick PMMA films containing different percentages of Disperse Red 1; however, the recorded holograms were usually unstable due to rotational diffusion of the azochromophores within the host matrix (Bian and Kuzyk,

2002; Ono *et al.*, 2001). An additional shortcoming of guest–host systems is that they can yield macroscopic segregation of chromophores from the polymer matrix depending on the host content.

As mentioned before, side-chain azopolymers can be used in preparing materials that register photoinduced birefringence. The influence of different polymer components and chromophore substituents has been thoroughly investigated (Natansohn and Rochon, 2002). For example, the length of the flexible spacer linking the chromophore to the polymer chain has been shown to significantly influence the photoinduced optical properties. The use of short spacers usually leads to amorphous polymers with low photoinduced birefringence values. This is ascribed to lack of mobility of the chromophore because it is tightly linked to the polymeric main chain, hindering azo reorientation. Increasing the length of the spacer generally leads to liquid crystalline phases, which usually result in higher photoinduced birefringence values (Matharu *et al.,* 2007). The flexible spacer favors decoupling of the movement of the azobenzene and main chain, allowing an efficient light-induced orientation of the chromophores (Labarthet *et al.,* 2000). In addition, the interactions between neighboring rod shaped mesogenic molecules usually promote liquid crystallinity that stabilize the orientation and even lead to amplification of the photoinduced order in darkness, through the so-called thermotropic effect (Kidowaki *et al.*, 2000; Zegber *et al.*, 2002).

As an example of side-chain liquid crystalline azopolymers, Hvilsted and co-workers described an interesting series of azobenzene homopolymers based on a polyester main chain (Holme *et al.*, 1996; Hvilsted *et al.,* 1995) in which two variable flexible spacers (main-chain and side-chain spacer) were introduced to the polymeric structure (see Fig. 16.5). An extensive exploration of the influence of these parameters, as well as the azobenzene substituents and the molecular weight in the photoinduced response, identified materials that, despite their low T_g, (sometimes below room temperature) can record large and stable photoinduced birefringence with a fast optical response. The interplay between photoinduced movements of the flexible polymeric segments, which also affect the polymeric main chain, and the liquid crystalline interactions between azo chromophores, is an aspect of these materials that merits much further study. As an example, irradiation of these materials with 488 nm linearly polarized blue light led to the usual preferential orientation of the chromophores, which was also transferred to the polymeric main-chain segments as demonstrated by infrared spectroscopy in deuterated systems (Kulinna *et al.,* 1998). This coupling between mesogens and the main chain leads to memory effects in the photoinduced birefringence. Large photoinduced birefringence values can be erased by short annealing times above the clearing temperature of the system, that randomizes the azomesogen orientation. However, cooling down to the mesophase leads to a recovery of



16.14 Azobenzene side-chain polymethacrylates: (a) typical structures of azopolymethacrylates and (b) example of azotolane-containing polymethacrylate reported by Ikeda and co-workers (Okano *et al.,* 2006b).

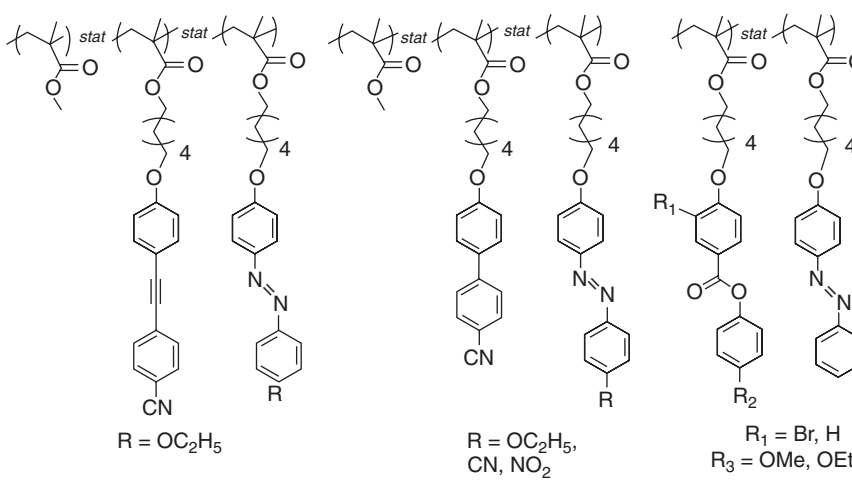
birefringence due to reorganization of the chomophores, which is guided by the orientation of the main chain (Han *et al.*, 2001).

Side-chain homopolymers based on poly(meth)acrylic derivatives (Fig. 16.14a) have been also widely studied (Andruzzi*et al.*, 1999; Rodríguez *et al.*, 2005, 2006). When a suitable flexible spacer mediates between the main chain and the chromophore, these side chain polymers tend to exhibit thermotropic liquid crystalline properties, which favor cooperative chromophores motion during photoorientation and lead to more stable anisotropic optical properties. The azobenzene chromophore strongly influences a material's photoresponse. As a representative example, Ikeda

and co-workers have prepared an interesting series of polymethacrylates containing an azobenzene group connected to a tolane moiety (azotolane), which increases the overall anisotropy of the molecular polarizability, and they have studied the positive influence of this moiety on the photoresponse and photoinduced properties (Fig. 16.14b) (Okano *et al.*, 2006a, b, c).

Dilution of the azobenzene content is required for volume holographic recording. For this purpose, statistical polymers composed of azobenzene and non-absorbing monomers like methyl methacrylate (in the case of copolymers prepared by chain polymerization) have been prepared by different research groups (Forcén *et al.*, 2007b; Lee *et al.*, 2006b). However, the main drawback of this approach for storage applications is the low values and stability of the photoinduced birefringence, due to the lack of cooperative interactions between the azobenzene units that are randomly distributed along the polymeric main chain.

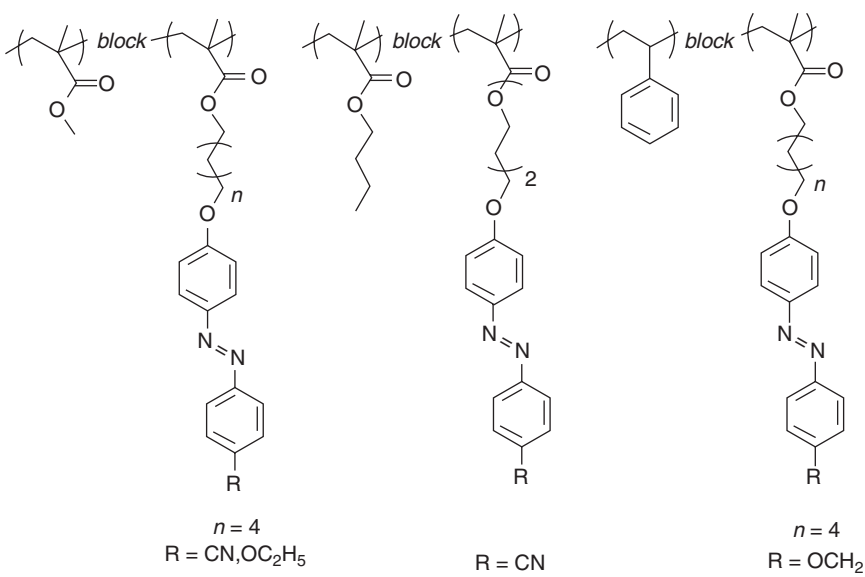
An alternative strategy, also based on statistical copolymerization, is the use of non-absorbing mesogenic monomers for copolymerization with azobenzene monomers (Fig. 16.15). Bieringer and co-workers prepared a series of side-chain methacrylic statistical copolymers with different percentages of azobenzene chromophore and a second mesogenic phenyl ester unit, which does not absorb light at the recording wavelength (Bieringer *et al.*, 1995). The presence of these mesogenic groups does not contribute to absorption in the same wavelength as azobenzene, but they can be oriented through cooperative motions and so increase the magnitude and stability of the photoresponse. However, the liquid crystalline behavior of these materials can result in light scattering. The formation of liquid crystalline phases in



16.15 Examples of statistical copolymers with azobenzene and mesogenic monomers.

these materials can be prevented by quenching the film from the isotropic state to RT (below T_g), leading to a metastable amorphous state, thus reducing the inherent light scattering of liquid crystalline phases that precludes their use in several optical applications. Volume holograms were recorded in thick films of these materials with moderate writing energies and times in the order of seconds. This approach has also been explored using other mesogenic moieties like tolane or biphenyl (Ishiguro *et al.*, 2007; Saishoji *et al.*, 2007). Multiple gratings were recorded in these materials. More than 50 volume gratings were multiplexed with a large dynamic range (8.9), demonstrating the potential of these materials in optical storage applications. However, even if thick films with low absorption and good optical response have been prepared, the liquid crystalline character of this type of copolymer can result in light scattering.

As mentioned above, one method of diluting azo content is the preparation of BCs combining a photoresponsive block containing azobenzene units with a non-absorbing polymeric block (Fig. 16.16). BCs self-organize into different microstructures (lamellar, spherical, cylindrical, etc.) depending on block length and macromolecular composition. The advantage of this microsegregation is that azobenzene moieties are confined in nanometric regions, so cooperative interactions among chromophores can be preserved to some extent. In addition, due to the size of these domains, which are usually much smaller than the recording wavelength, light scattering can be significantly reduced in these materials in comparison to their homopolymer



16.16 Examples of azobenzene BCs.

counterparts. Most of the reported azobenzene BCs have a non-absorbing block that is either PMMA (Forcén *et al*., 2007a; Gimeno *et al*., 2009) or PS (Chen *et al*., 2011; Häckel *et al*., 2005a). The photoresponsive block can be composed of an azobenzene side-chain polymer or a random copolymer containing other mesogenic groups (Forcén *et al*. 2007a; Häckel *et al.*, 2005a; Yu *et al.*, 2008). The influence of microdomain morphology on the photoinduced response in these materials, as well as the possibility of registering stable holograms, has been thoroughly investigated. For example, a series of BCs based on a PMMA block and an azomethacrylate block were prepared and their photoresponse to 488 nm linearly polarized light evaluated (Gimeno *et al.*, 2009). BCs with molecular weight Mn in the range of 18000 to 27000 and different volume fractions of the constituent blocks led to lamellar and spherical microstructures. Photoinduced birefringence per azobenzene unit in BCs with lamellar microstructures was revealed to be similar to that attained in the homopolymer. Lower and less stable values were obtained from BCs with spherical morphology, which could be related to confinement effects. Further investigation into homologous BC systems with higher molecular weights (Mn = 92 000),which are segregated into larger azobenzene-containing spheres, led to a similar photoinduced birefringence per azobenzene unit to that reached in lamellar systems (and therefore to the homopolymer) that the photoresponse is conditioned not only by the type of morphology but also by the size of the segregated nanoobjects (Berges *et al.,* 2012a).

A simple strategy to further dilute the azobenzene content while maintaining control of the azobenzene environment can be achieved by blending BCs with amorphous polymers that are non-absorbing at the recording wavelength, for example the same polymer as the non-absorbing block. Selecting an appropriate molecular weight makes it possible to obtain well-defined microsegregated domains of photoresponsive materials within a non-absorbing matrix without macrosegregation (Audorff *et al.*, 2010; Berges *et al*., 2012a; Breiner *et al.*, 2007; Forcén *et al.*, 2008; Häckel *et al.*, 2005b, 2007). For example, the addition of PMMA of the appropriate molecular weight to a polymethacrylic BC showing lamellar microdomains resulted in the formation of microspheres. The photoresponse of this diluted system is lower and less stable than the original BC. Homologous BCs with larger molecular weights and spherical morphologies have also been diluted with PMMA (Berges *et al.*, 2012a). In this case the spherical morphology is retained, as well as the magnitude of the photoresponse. This has enabled the preparation of films hundreds of microns thick with reduced absorption of these blends to record and multiplex volume holograms with light pulses of 10 ms (Berges *et al.*, 2013a).

More recently, Alcalá and co-workers reported direct blending of a side-chain liquid crystal azobenzene homopolyester with PMMA, diluting the

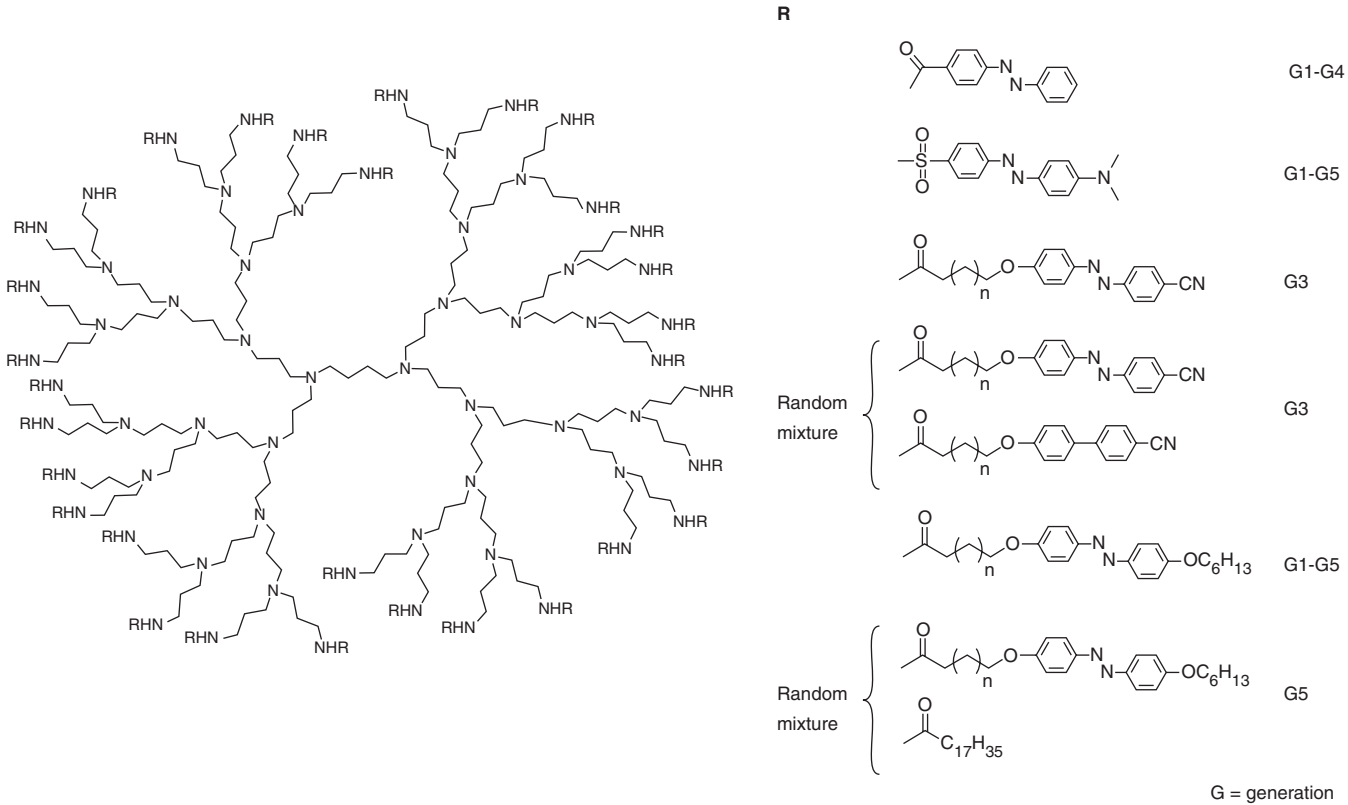
final azo content down to 0.1 wt%. Thick films of this material displayed good optical quality, and stable volume holograms were multiplexed by using low energy light pulses and reducing the duration to 2 ms (Berges *et al.,* 2013b).

16.6 Alternative macromolecular architectures for the design of azopolymers

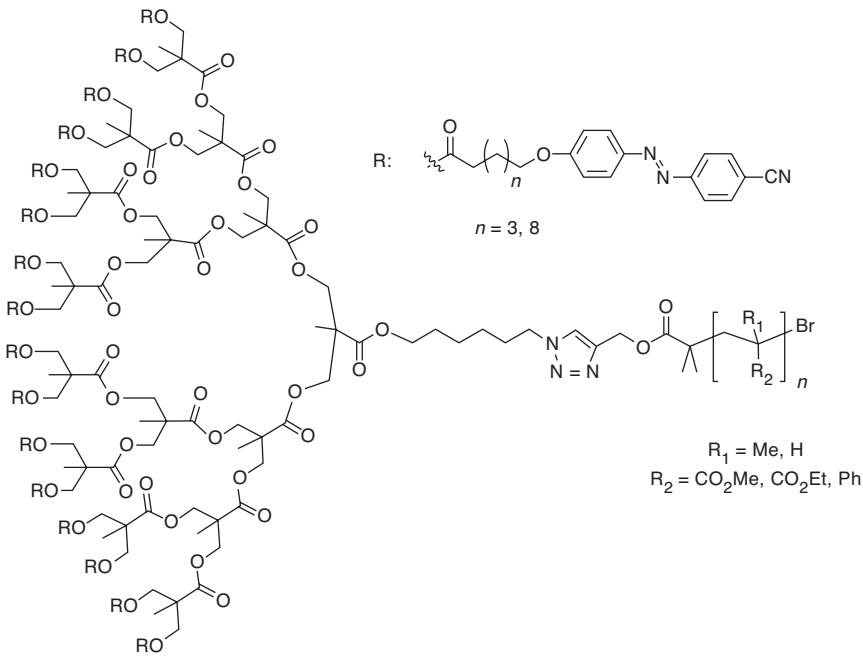
In recent years, new smart polymers have been proposed with different macromolecular architectures to the linear polymers described so far. Dendritic architectures are of particular interest due to their unusual and well-defined structure that could lead to novel or unexpected properties (Archut *et al*., 1998; Astruc *et al.,* 2010; Grayson and Fréchet, 2001). Dendrimers are highly branched monodisperse macromolecules at the nanoscale, which can be modified for the incorporation of azobenzenes at specific locations in the dendritic structure. The azobenzene units can be part of the core moiety, located at the branching points or appended to the periphery of the dendritic structure (Deloncle and Caminade, 2010). This last approach has been explored by several groups for the preparation of azodendrimers, mainly using commercially available poly(propyleneimine) dendritic cores. The functionalization of the dendritic scaffold was mainly accomplished by reaction of the amine peripheral groups of the dendrimer with the azobenzene moieties generally containing terminal activated carboxylic groups, such as the examples collected in Fig. 16.17 (Alcalá *et al.*, 2007).

Dendrimers offer a clear advantage in the design of smart polymers in comparison to linear polymers. Precise control over the number of chromophoric units per macromolecule is possible which, in turn, can be varied with dendrimer generation. This feature can also be exploited in the design of new BCs with linear–dendritic macromolecular architectures (Wurm and Frey, 2011). Azobenzene linear–dendritic BCs are composed of a non-absorbing linear block linked to a dendron functionalized with photoresponsive moieties. These materials combine the good properties of BCs, segregation ability and chromophore dilution, with control over the number of chromophore moieties in each macromolecular chain. Through this approach, several photoresponsive linear–dendritic BCs with azobenzene units have been prepared using dendrons derived from 2,2-bis(hydroxymethyl)propionic acid (bis-MPA) and PEG, PMMA, poly(ethyl methacrylate) or PS as linear segments (see examples in Fig. 16.18) (Blasco *et al.*, 2012; del Barrio *et al.*, 2009, 2010).

Preparation of conventional linear–dendritic BCs can be achieved through three basic strategies:(i) 'chain-first' route, (ii) 'dendron-first' route and (iii) coupling of the preformed blocks. The chain-first route involves synthesizing

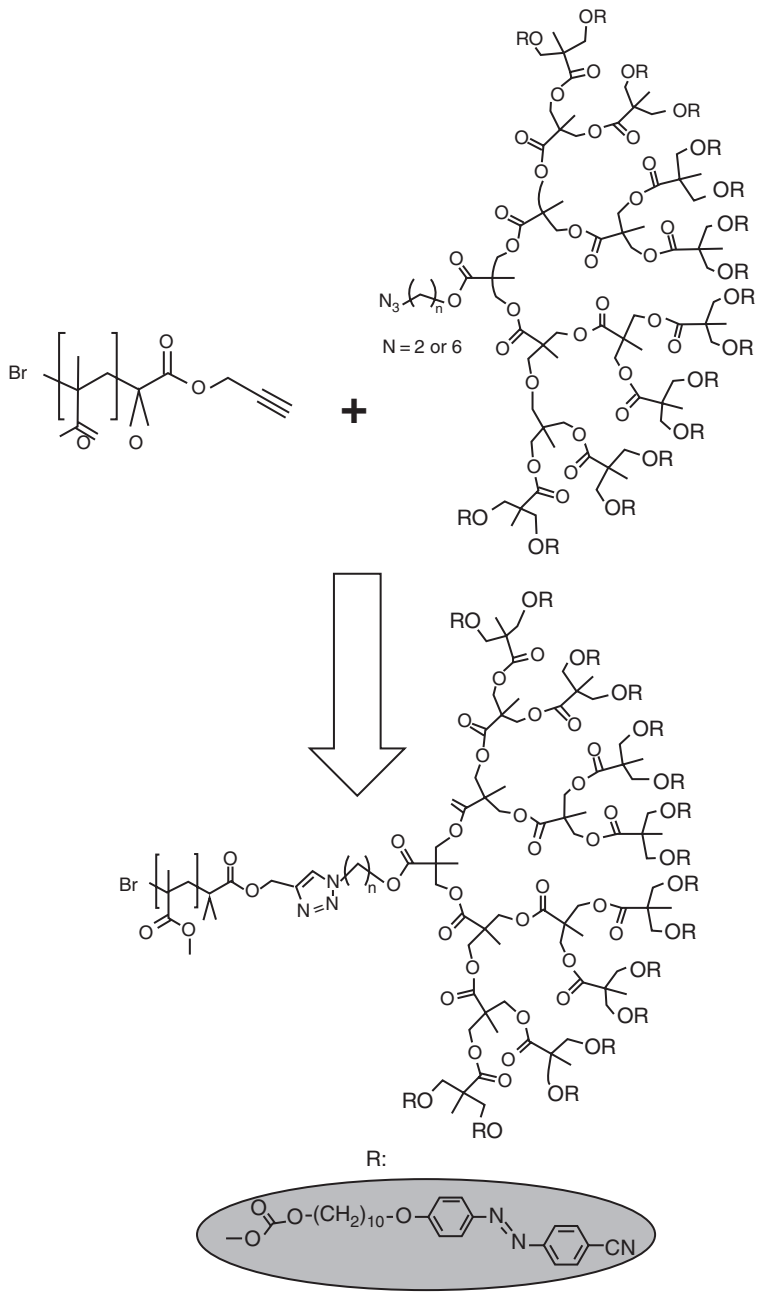


16.17 Azobenzene functionalized poly(propyleneimine) dendrimers (G4 dendrimer generation is represented on the left).



16.18 Azobenzene linear–dendritic BCs with a dendron of fourth generation derived from bis-MPA.

a terminally functional polymer followed by a divergent dendron construction (Iyer *et al.*, 1998; van Hest *et al.*, 1996). The dendron-first route requires the preparation of a dendron functionalized at the focal point which is used as a macroinitiator for polymerization of the linear block (Leduc *et al.*, 1996; Matyjaszewski *et al.*, 1996). However, coupling of the blocks is the most versatile synthetic approach. This coupling strategy involves synthesis of the linear and dendron segments, followed with their coupling by a chemical reaction of functional groups located at the end of the linear block and the focal point of the dendron. This approach relies on the use of highly effective click chemistry reactions. In this case CuAAC was chosen for the synthesis of the first azo BCs with linear–dendritic architectures (Blasco *et al.*,2012; del Barrio *et al.*, 2009, 2010). Dendrons derived from bis-MPA are an excellent platform for this purpose. They can be easily functionalized at the focal point with an azide group due to the presence of a carboxylic group at the core, as well as with azobenzene moieties at the periphery due to the presence of multiple hydroxyl groups. On the other hand, preparation of the linear polymeric chain can be carried out by ATRP using an initiator containing an alkyne group. In the final step, both blocks are coupled, as described in Fig. 16.19. A slight excess of the linear block (due to its lower



16.19 Click coupling of a PMMA linear block and an azo functionalized bis-MPA dendron.

cost) is used to ensure a complete reaction of the dendron and can be easily eliminated using polymeric resins functionalized with azide groups. Despite good control over the polymeric architecture in linear–dendritic BCs, these materials show photoresponse properties inferior to those of linear–linear analogues.

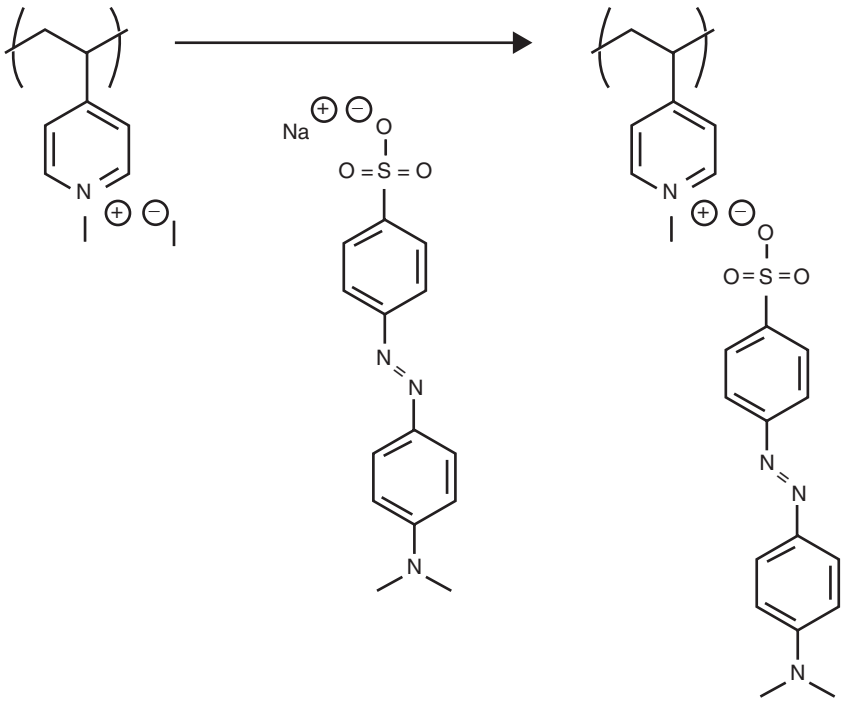
Other complex architectures, such as azobenzene star polymers (Angiolini *et al.,* 2006) and molecular brushes (Lee *et al.*, 2006a), have also been prepared, although their optical data storage capabilities have not yet been explored. Advances in these new macromolecular architectures are generally based on the combination of CRP and click chemistry reactions as a synthetic tool for achieving more complex smart polymers.

All the photoresponsive materials described above are based on covalent structures. However, the principles of supramolecular chemistry can be successfully applied to the design of non-covalent functional materials (de Greef *et al.*, 2008, 2009; Yagai and Kitamura, 2008). A clear advantage of supramolecular smart polymers stems from their easy preparation when compared with their covalent counterparts. In the case of supramolecular azopolymers, their preparation usually implies linking of the photochromic moieties to the macromolecular structure by means of non-covalent bonding.

For instance, Bazuin and co-workers (Zhang *et al.*, 2007a) applied this approach in describing polyelectrolyte ionic complexes analogous to side-chain azopolymers. Methyl orange was used as a photochromic moiety to functionalize poly(*N*-methylpyridine), via an anion-exchange procedure, which allows a high doping level of a polymeric matrix (Fig. 16.20) (Priimagi *et al.*, 2005, 2007).

The ionic functionalization of dendritic and hyperbranched polymers (Fig. 16.21) (Marcos *et al.*, 2008) and codendrimers (Hernández-Ainsa *et al.*, 2011) has also been recently described. The ionic complexes can be easily prepared by mixing chromopohoric carboxylic acid and amine-ended dendrimers or random hyperbranched polymers in a common solvent. Proton transfer from the carboxylic acid to amine groups is confirmed by spectroscopic techniques. However, one should take into account that amide bonds can be formed upon heating during the processing or manipulation of these materials, which alters the chemical structure. This undesired reaction can be minimized or prevented by transforming the terminal primary (or secondary) amino groups into tertiary amino groups (Chen *et al.*, 2006), for instance by previous methylation of the peripheral groups.

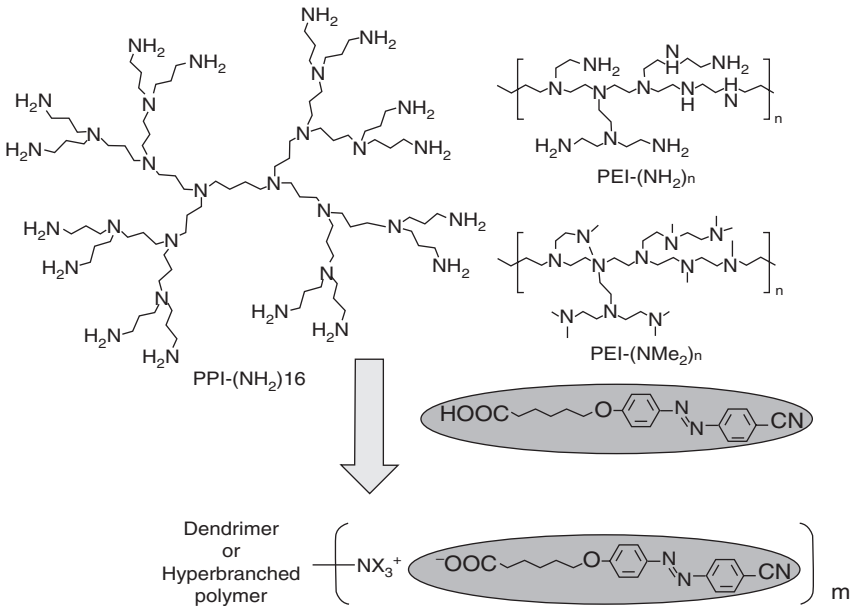
Another attractive supramolecular approach is the self-assembly of polymers and chromophores by H-bonding. This non-covalent and directional bonding offers high versatility for the preparation of functional smart



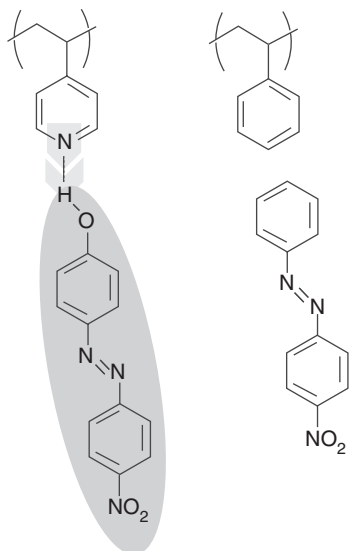
16.20 Preparation of ionic photochromic polymers by ion exchange.

polymers, since the supramolecular recognition of H-donor and H-acceptor molecules gives rise to reversible materials with improved properties. Poly(vinylpyridine) derivatives are the preferred polymeric platform in the design of supramolecular polymers, mainly due to their commercial availability. In the field of photoresponse polymers for optical storage, Ikkala and co-workers demonstrated the enhanced photoinduced birefringence with high temporal stability of a supramolecular system obtained from poly(4-vinylpyridine) and 4-nitro-4′-hydroxyazobenzene in comparison to an analogous guest–host system formed by poly(styrene) and 4-nitroazobenzene (Fig. 16.22) (Priimagi *et al.*, 2008). The origin of this improved performance is in the self-assembly and intermolecular interactions between the pyridine rings and the chomophore.

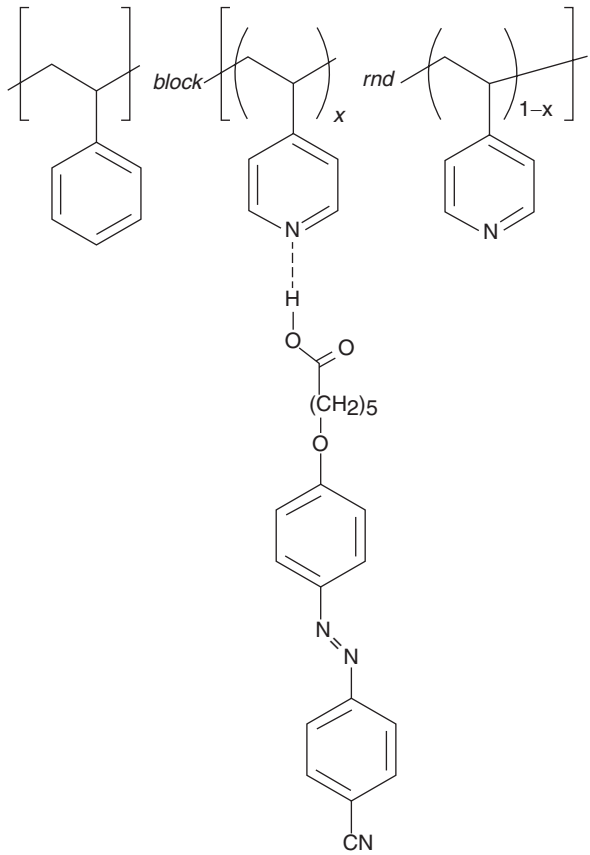
The same strategy has been used very recently to prepare BCs containing a low percentage of azobenzene moieties, from commercial poly(styrene)-*block*-poly(4-vinylpyridine) copolymers. A chromophore with a carboxylic group has been used in the preparation of liquid crystalline BCs. It should be noted that these systems are only homogeneous when the percentage of



16.21 Ionic functionalization of dendrimers and random hyperbranched polymers.



16.22 Hydrogen-bonded azopolymer described by Ikkala and co-workers (left) and analogous model without H-bonding (right).



16.23 Supramolecular BCs with carboxy terminated azobenzene moieties.

H-bonded pyridine repeating units is equal to or lower than 50% ($x \leq 0.5$) (Fig. 16.23). Despite the reduced azo content in some of these systems, photoresponse was close to that achieved by covalent BCs. Nevertheless, in contrast to the synthesis of covalent BCs, this supramolecular approach is a more versatile and less laborious strategy for accessing materials with low chromophore content (del Barrio *et al.*, 2013).

16.7 Conclusion

Smart photoaddressable polymers containing azobenzene units have been actively investigated over more than two decades as media for optical data storage. Information can be recorded in these materials, through the effect of

polarized light, as local changes in birefringence. This information can later be erased by optical means, making these materials potential candidates for rewritable optical storage. Among the different strategies for incorporating azobenzenes into the polymeric architecture, covalent linking of the photochromic moiety to the polymeric chain as a pendant group has been by far the most widely studied. The promesogenic character of the azobenzene unit in these systems can lead to liquid crystallinity, usually resulting in large and stable values of photoinduced birefringence.

Despite past efforts to explore the potential of these materials as thin layers for bit-wise 2D optical recording, research has more recently focused on the use of these photoresponsive systems as rewritable volume holographic media. Besides high sensitivity and stability of the photoinduced birefringence, additional requirements, such as good optical quality and low absorption at the recording wavelengths, are needed in this case. Copolymerization with non-absorbing monomers has been investigated as a strategy to dilute the azobenzene content, thereby obtaining thick films with suitable absorption for volume holography. In particular, the advent of controlled polymerizations and the application of click chemistry to polymer science has allowed the synthesis of well-defined block copolymer architectures with a photoresponsive azobenzene block and a non-absorbing one. This is a demonstrably effective strategy for diluting the azobenzene content while keeping the cooperative interaction between azobenzene mesogens, leading to efficient and stable holograms in thick films. Further dilution of the azo content has been achieved by blending these photoresponsive BCs with a non-absorbing homopolymer. Actually, the effective multiplexing of several holograms in transparent thick films of such blends has shown the potential of these materials as volume holographic media.

Although acceptable results have been achieved with some of these systems, there is still room for improvement. The search for new photoresponsive materials has led to the investigation of more complex architectures, such as dendrimers or linear–dendritic BCs, but the results obtained so far show no improvement over those previously reported for analogous linear side-chain polymers. Research into photoresponsive materials is focused on the development of materials with better sensitivity that can easily be prepared and processed as good quality thick films while their photoresponse is kept or improved. Photoaddressable supramolecular polymers may offer an interesting and simple alternative to covalent systems. The direct blending of an azobenzene photoresponsive homopolymer and a non-absorbing one has recently been revealed as a simple and effective method for the preparation of materials with high azobenzene dilution, suitable for the preparation of thick films for volume holography.

16.8 References

Adams, J. and Gronski, W. (1989) 'LC Side Chain AB-Block copolymers with an Amorphous A-Block and a Liquid Crystalline B-Block', *Makromol. Chem. Rapid Commun.,* **10**, 553.

Alcalá, R., Giménez, R., Oriol, L., Piñol, M., Serrano, J. L., Villacampa, B. and Viñuales, A. I. (2007) 'Synthesis, characterization, and induction of stable anisotropy in liquid crystalline photo-addressable PPI dendrimers', *Chem. Mater.,* **19**, 235–246.

Altomare, A., Ciardelli, F., Tirelli, N. and Solaro, R. (1997) '4-Vinylazobenzene: Polymerizability and photochromic properties of its polymers', *Macromolecules*, **30**, 1298–1303.

Andruzzi, L., Altomare, A., Ciardelli, F., Solaro, R., Hvilsted, S. and Ramanujam P.S. (1999) 'Holographic gratings in azobenzene side-chain polymethacrylates' *Macromolecules*, **32**, 448–454.

Angiolini, L., Benelli, T., Giorgini, L., Mauriello, F., Salatelli, E., Bozio, R., Daurù, A. and Pedron, D. (2007) 'Synthesis, chiroptical properties and photoinduced birefringence of optically active methacrylic copolymers bearing side-chain bisazoaromatic moieties', *Eur. Polym. J.,* **43**, 3550–3561.

Angiolini, L., Benelli, T., Giorgini, L., Salatelli, E., Bozio, R., Dauru, A. and Pedron, D. (2006) 'Improvement of photoinduced birefringence properties of optically active methacrylic polymers through copolymerization of monomers bearing azoaromatic moieties'. *Macromolecules*, **39**, 489–497.

Archut, A., Vögtle, F., De Cola, L., Azzellini, G. C., Balzani, V., Ramanujam, P. S. and Berg, R. H. (1998) 'Azobenzene-functionalized cascade molecules: Photoswitchable supramolecular systems', *Chem. Eur. J.*, **4**, 699.

Astruc, D., Boisselier, E. and Ornelas, C. T. (2010) 'Dendrimers designed for functions: From physical, photophysical, and supramolecular properties to applications in sensing, catalysis, molecular electronics, photonics, and nanomedicine', *Chem. Rev.*, **110**, 1857–1959.

Audorff, H., Kreger,K., Walker, R., Haarer, D., Kador L. and Schmidt, H. –W. (2010) 'Holographic gratings and data storage in azobenzene-containing block copolymers and molecular glasses', *Adv. Polym. Sci.,* **228**, 59–121.

Bandara, H. M. D. and Burdette, S. C. (2012) 'Photoisomerization in different classes of azobenzene', *Chem. Soc. Rev.,* **41**, 1809–1825.

Berges, C., Gimeno, N., Oriol, L., Piñol, M., Forcén, P., Sánchez, C. and Alcalá, R. (2012a) 'Photoinduced optical anisotropy in azobenzene containing block copolymer–homopolymer blends. Influence of microstructure and molecular weight', *Eur. Polym. J.,* **48**, 613.

Berges, C., Javakhishvili, I., Hvilsted, S., Sánchez-Somolinos, C. and Alcalá, R. (2012b) 'Photoresponsive Azopolyester-PMMA block copolymers obtained by combination of ATRP, polycondensation and "click" chemistry', *Macromol. Chem. Phys.*, **213**, 2299–2310.

Berges, C., Javakhishvili, I., Hvilsted, S., Sánchez-Somolinos, C. and Alcalá, R. (2013b) 'Holographic storage and multiplexing in azopolyester blends using low energy pulses down to 2 ms', *Appl. Phys. Lett.,* **102**, 193303 1–193303 4.

Berges, C., Oriol, L., Piñol, M., Sánchez-Somolinos, C. and Alcalá, R. (2013a) 'Blends of an azomethacrylic block copolymer for volume holographic storage using low energy 10 ms light pulses', *Opt. Mat.,* **35**, 1095–1098.

Berkovic, G., Krongauz, V. and Weiss, V. (2000) 'Spiropyrans and spirooxazines for memories and switches', *Chem. Rev.*, **100**, 1741–1754.

Bian, S. and Kuzyk, M. G. (2002) 'Real-time holographic reflection gratings in volume media of azo-dye-doped poly(methyl methacrylate)', *Opt. Lett.,* **27**, 1761–1763.

Bieringer, T., Wuttke, R., Haarer, D., Geßner, U. and Rübner, J. (1995) 'Relaxation of holographic gratings in liquid-crystalline side chain polymers with azo chromophores', *Macromol. Chem. Phys.,* **196**, 1375–1390.

Blasco, E., del Barrio, J., Piñol, M., Oriol, L., Berges, C., Sánchez, C. and Alcalá, R. (2012) 'Azobenzene-containing linear–dendritic block copolymers prepared by sequential ATRP and click chemistry', *Polymer*, **53**, 4604–4613.

Breiner, T., Kreger, K., Hagen, R., Häckel, M., Kador, L., Müller, A. H. E., Kramer, E. J. and Schmidt, H.-W. (2007) 'Blends of poly(methacrylate) block copolymers with photoaddressable polymers', *Macromolecules*, **40**, 2100–2108.

Bruder, F. -K., Hagen, R., Rölle, T., Weiser M. -S. and Fäcke, T. (2011) 'From the surface to volume: Concepts for the next generation of optical-holographic data-storage materials', *Angew. Chem. Int. Ed.*, **50**, 4552–4573.

Chen, F. S., LaMacchia, J. T. and Fraser, D. B. (1968) 'Holographic storage in lithium niobate', *Appl. Phys. Lett.,* **13**, 223–225.

Chen, W., Wei, X., Balazs, A. C., Matyjaszewski, K. and Russell, T. P. (2011) 'Phase behavior and photoresponse of azobenzene-containing polystyrene-block-poly(n-butyl methacrylate) block copolymers', *Macromolecules,* **44**, 1125–1131.

Chen, Y., Shen, Z., Gehringer, L., Frey, H. and Stiriba, S.-E. (2006) 'Supramolecular thermotropic liquid crystalline materials with nematic mesophase based on methylated hyperbranched polyethylenimine and mesogenic carboxylic acid', *Macromol. Rapid Comm.,* **27**, 69–75.

Cojocariu, C. and Rochon, P. (2005) 'Thermotropic side-chain liquid crystalline copolymers containing both mono- and bisazobenzene mesogens: Synthesis and properties', *Macromolecules,* **38**, 9526–9538.

Colburn, W. S. and Haines, K. A. (1971) 'Volume hologram formation in photopolymer materials', *Appl. Opt.,* **10**, 1636–1641.

Cui, L., Zhao, Y., Yavrian, A. and Galstian, T. (2003) 'Synthesis of azobenzene-containing diblock copolymers using atom transfer radical polymerization and the photoalignment behavior', *Macromolecules,* **36**, 8246–8252.

Czapla, S., Ruhmann, R., Rübner, J., Zschuppe, V. and Wolff, D. (1993) 'New copolymethacrylates with azo-chromophores in the side chain', *Die Makromolekulare Chemie,* **194**(1), 243–250.

de Greef, T. F. A. and Meijer, E. W. (2008) 'Supramolecular polymers', *Nature*, **453**, 171–173.

de Greef, T. F. A., Smulders, M. M. J., Wolffs, M., Schenning, A. P. H. J., Sijbesma, R. P. and Meijer, E. W. (2009) 'Supramolecular polymerization', *Chem. Rev.*, **109**, 5687–5754.

del Barrio, J., Blasco, E., Oriol, L., Alcalá, R. and Sánchez-Somolinos, C. (2013) 'Diblock copolymer–azobenzene complexes through hydrogen bonding: Self-assembly and stable photoinduced optical anisotropy', *J. Polym. Sci. Part A: Polym. Chem.,* **51**(8), 1716–1725.

del Barrio, J., Oriol, L., Alcala, R. and Sanchez, C. (2009) 'Azobenzene-containing linear-dendritic diblock copolymers by click chemistry: Synthesis, characterization, morphological study, and photoinduction of optical anisotropy', *Macromolecules,* **42**, 5752–5760.

del Barrio, J., Oriol, L., Alcala, R. and Sanchez, C. (2010) 'Photoresponsive poly(methyl methacrylate)-b-azodendron block copolymers prepared by ATRP and click chemistry', *J. Polym. Sci. Polym. Chem.,* **48**, 1538–1550.

Deloncle, R. and Caminade, A.-M. (2010) 'Stimuli-responsive dendritic structures: The case of light-driven azobenzene-containing dendrimers and dendrons', *J. Phochem. Photobiol. C: Photochem. Rev.,* **11**, 25–45.

Deng, W., Albouy, P.-A., Lacaze, E., Keller, P., Wang, X. and Li, M.-H. (2008) 'Azobenzene-containing liquid crystal triblock copolymers: Synthesis, characterization, and self-assembly behavior', *Macromolecules,* **41**, 2459–2466.

Forcén, P., Oriol, L., Sanchez, C., Alcalá, R., Hvilsted, S., Jankova, K. and Loos, J. (2007a) 'Synthesis, characterization and photoinduction of optical anisotropy, in liquid crystalline diblock azo-copolymers', *J. Polym. Sci. Polym. Chem.,* **45**, 1899–1910.

Forcén, P., Oriol, L., Sánchez, C., Rodriguez, F. J., Alcalá, R., Hvilsted, S. and Jankova, K. (2007b) 'Methacrylic azopolymers for holographic storage: A comparison among different polymer types', *Eur. Polym. J.*, **43**, 3292–3300.

Forcén, P., Oriol, L., Sanchez, C., Rodríguez, F. J., Alcala, R., Hvilsted S. and Jankova, K. (2008) 'Volume holographic storage and multiplexing in blends of PMMA and a block methacrylic azopolymer using 488 nm light pulses in the range of 100 ms to 1 s', *Eur. Polym. J.*, **44**, 72–78.

Frenz, C., Fuchs, A., Schmidt, H.-W., Theissen, U. and Haarer, D. (2004) 'Diblock copolymers with azobenzene side-groups and polystyrene matrix: Synthesis, characterization and photoaddressing', *Macromol. Chem. Phys.,* **205**, 1246–1258.

Gao, J., Sun, Y., Zhou, J., Zheng, Z., Chen, H., Su, W. and Zhang, Q. (2007) 'Preparation of Ag nanoparticles termini-protected side-chain liquid crystalline azobenzene polymers by RAFT polymerization', *J. Polym. Sci. Polym. Chem.,* **45**, 5380.

Gimeno, S., Forcén, P., Oriol, L., Pinol, M., Sanchez, C., Rodriguez, F. J., Alcala, R., Jankova, K. and Hvilsted, S. (2009) 'Photoinduced optical anisotropy in azobenzene methacrylate block copolymers: Influence of molecular weight and irradiation conditions', *Eur. Polym. J.,* **45**, 262–271.

Grayson, S. M. and Fréchet, J. M. J. (2001) 'Convergent dendrons and dendrimers: From synthesis to applications', *Chem. Rev.,* **101**, 3819–3868.

Häckel, M., Kador, L., Kropp, D., Frenz, C. and Schmidt, H. W. (2005a) 'Holographic gratings in diblock copolymers with azobenzene and mesogenic side groups in the photoaddressable dispersed phase', *Adv. Funct. Mater.,* **15**, 1722–1727.

Häckel, M., Kador, L., Kropp, D., Frenz C. and Schmidt, H. W. (2005b) 'Holographic information storage in azobenzene-containing diblock copolymers', *Proc. SPIE,* **5939**, 593908 1–593908 10.

Häckel, M., Kador, L., Kropp, D. and Schmidt, H. W. (2007) 'Polymer blends with azobenzene-containing block copolymers as stable rewritable volume holographic media', *Adv. Mater.,* **19**, 227–231.

Hagen, R. and Bieringer, T. (2001) 'Photoaddressable polymers for optical data storage', *Adv. Mater.*, **13**, 1805–1810.

Hallensleben, M. and Weichart, B. (1989) 'Photoresponsive polymers', *Polym. Bull.*, **22**, 553–556.

Han, M., Kidowaki, M. Ichimura, K., Ramanujam P. S. and Hvilsted S. (2001) 'Influence of structures of polymer backbones on cooperative photoreorientation behavior of p-cyanoazobenzene side chains', *Macromolecules,* **34**, 4256–4262.

Hernández-Ainsa, S., Alcalá, R., Barberá, J., Marcos, M., Sánchez, C. and Serrano, J.L. (2011) 'Ionic azo-codendrimers: Influence of the acids contents in the liquid crystalline properties and the photoinduced optical anisotropy', *Eur. Polym. J.,* **37**, 311–318.

Holme, N. C. R., Ramanujam, P. S. and Hvilsted, S. (1996) 'Photoinduced anisotropy measurements in liquid-crystalline azobenzene side-chain polyesters', *Appl. Opt.,* **35**, 4622–4627.

Hvilsted, S., Andruzzi, F., Kulinna, C., Siesler, H. W. and Ramanujam, P. S. (1995) 'Novel side-chain liquid crystalline polyester architecture for reversible optical storage', *Macromolecules,* **28**, 2172–2183.

Ishiguro, M., Sato, D., Shishido, A. and Ikeda, T. (2007) 'Bragg-type polarization gratings formed in thick polymer films containing azobenzene and tolane moieties†', *Langmuir,* **23**, 332–338.

Iyer, J., Fleming, K. and Hammond, P. T. (1998) 'Synthesis and solution properties of new linear-dendritic diblock copolymers', *Macromolecules,* **31**, 8757–8765.

Jin, M., Xin Yang, Q., Lu, R., Hua Xu, T. and Ying Zhao, Y. (2004) 'Syntheses of bisazo-containing polymethacrylates using atom transfer radical polymerization and the photoalignment behavior', *J. Polym. Sci. Polym. Chem.,* **42**, 4237–4247.

Kadota, S., Aoki, K., Nagano, S. and Seki, T. (2005) 'Photocontrolled microphase separation of block copolymers in two dimensions', *J. Amer. Chem. Soc.,* **127**, 8266–8267.

Kang, E.-H., Liu, X., Sun, J. and Shen, J. (2006) 'Robust ion-permselective multilayer films prepared by photolysis of polyelectrolyte multilayers containing photo-cross-linkable and photolabile groups', *Langmuir,* **22**, 7894–7901.

Kidowaki, M., Fujiwara, T., Morino, S.Y., Ichimura, K. and Stumpe, J. (2000) 'Thermal amplification of photoinduced optical anisotropy of p-cyanoazobenzene polymer films monitored by temperature scanning ellipsometry', *J. Appl. Phys. Lett.,* **76**, 1377 (3 pages).

Kulinna, C., Hvilsted, S., Hendann, C., Siesler, H.W. and Ramanujam, PS (1998) 'Selectively deuterated liquid crystalline cyanoazobenzene side-chain polyesters. 3. Investigations of laser-induced segmental mobility by fourier transform infrared spectroscopy', *Macromolecules,* **31**, 2141–2151.

Labarthet, F.L., Freiberg, S., Pellerin, C., Pszolet, M., Natansohn, A. and Rochon, P. (2000) 'Spectroscopic and optical characterization of a series of azobenzene-containing side-chain liquid crystalline polymers', *Macromolecules,* **33**, 6815–6823.

Leduc, M. R., Hawker, C. J., Dao, J. and Frechet, J. M. J. (1996) 'Dendritic initiators for "living" radical polymerizations: A versatile approach to the synthesis of dendritic-linear block copolymers', *J. Amer. Chem. Soc.,* **118**, 11111–11118.

Lee, H.-i., Pietrasik, J. and Matyjaszewski, K. (2006a) 'Phototunable temperature-responsive molecular brushes prepared by ATRP', *Macromolecules,* **39**, 3914–3920.

Lee, M.-J., Jung, D.-H. and Han, Y.-K. (2006b) 'Photo-responsive polymers and their applications to optical memory', *Mol. Cryst. Liq. Cryst.,* **444**, 41–50.

Lenoble, C. and Becker, R.S. (1986) 'Photophysics, photochemistry, and kinetics of photochromic fulgides', *J. Phys. Chem*., **90**, 2651–2654.

Li, M.-H., Keller, P., Grelet, E. and Auroy, P. (2002) 'Liquid-crystalline polymethacrylates by atom-transfer radical polymerization at ambient temperature', *Macromol. Chem. Phys.,* **203**, 619.

Marcos, M., Alcalá, R., Barberá, J. n., Romero, P., Sánchez, C. and Serrano, J. L. (2008) 'Photosensitive ionic nematic liquid crystalline complexes based on dendrimers and hyperbranched polymers and a cyanoazobenzene carboxylic acid†', *Chem. Mater.,* **20**, 5209–5217.

Matharu, A.S., Jeeva, S. and Ramanujan, P.S. (2007) 'Liquid crystals for holographic optical data storage', *Chem. Soc. Rev.,* **36**, 1868–1880.

Matyjaszewski, K., Shigemoto, T., Frechet, J. M. J. and Leduc, M. (1996) 'Controlled/"living" radical polymerization with dendrimers containing stable radicals', *Macromolecules,* **29**, 4167–4171.

Natansohn A. and Rochon P. (2002) 'Photoinduced motions in Azo-containing polymers', *Chem. Rev.,* **102**, 4139–4175.

Natansohn, A., Rochon, P., Barrett, C. and Hay, A. (1995) 'Stability of photoinduced orientation of an azo compound into a high-T, polymer', *Chem. Mater.,* **7**, 1612–1615.

Nikolova L. and Todorov T. (1984) 'Diffraction efficiency and selectivity of polarization holographic recording', *Opt. Acta,* **31**, 579–588.

Nuyken, O. and Weidner, R. (1986) 'Graft and block copolymers via polymeric azo initiators', *Adv. Polym. Sci.,* **73/74**, 145–199.

Okano, K., Shishido, A. and Ikeda, T. (2006a) 'Photochemical phase transition behavior of highly birefringent azotolane liquid-crystalline polymer films: Effects of the position of the tolane group and the donor–acceptor substituent in the mesogen', *Macromolecules,* **39**, 145–152.

Okano, K., Shishido, A. and Ikeda, T. (2006b) 'An azotolane liquid-crystalline polymer exhibiting extremely large birefringence and its photoresponsive behavior', *Adv. Mater.,* **18**, 523–527.

Okano, K., Tsutsumi, O., Shishido, A. and Ikeda, T. (2006c) 'Azotolane liquid-crystalline polymers: Huge change in birefringence by photoinduced alignment change', *J. Amer. Chem. Soc.,* **128**, 15368–15369.

Ono, H., Kowatari, N. and Kawatsuki, N. (2001) 'Holographic grating generation in thick polymer films containing azo dye molecules', *Opt. Mater.,* **17**, 387–394.

Priimagi, A., Cattaneo, S., Ras, R. H. A., Valkama, S., Ikkala, O. and Kauranen, M. (2005) 'Polymer–dye complexes: A facile method for high doping level and aggregation control of dye molecules', *Chem. Mater.,* **17**, 5798–5802.

Priimagi, A., Kaivola, M., Rodriguez, F. J. and Kauranen, M. (2007a) 'Enhanced photoinduced birefringence in polymer-dye complexes: Hydrogen bonding makes a difference', *Appl. Phys. Lett.,* **90**, 121103–3.

Priimagi, A., Vappavuori, J., Rodriguez, F. J., Faul, C.F.J., Heino, M.T., Ikkala, O., Kauranen, M. and Kaivola, M. (2008) 'Hydrogen-bonded polymer-azobenzene complexes: Enhanced photoinduced birefringence with high temporal stability through interplay of intermolecular interactions', *Chem. Mater.,* **20**, 6358–6363.

Ringsdorf, H. and Schmidt, H.-W. (1984) 'Electro-optical effects of azo dye containing liquid crystalline copolymers', *Makromol. Chem.,* **185**, 1327–1334.

Rodríguez, F. J., Sanchez, C., Villacampa, B., Alcalá, R., Cases, R., Millaruelo, M. and Oriol, L. (2005) 'Fast and stable recording of birefringence and holographic gratings in an azo-polymethacrylate using a single nanosecond light pulse', *J. Chem. Phys.,* **123**, 204706.

Rodríguez, J. F., Sánchez, C., Villacampa, B., Alcalá, R., Cases, R., Millaruelo, M., Oriol, L. and Lörincz, E. (2006) 'Red light induced holographic storage in an azobenzene polymethacrylate at room temperature', *Opt. Mater.,* **28**, 480.

Ruhmann, R. (1997) 'Polymers for optical storage–photoresponsive polymers by structure variation in side-group polymethacrylates', *Polym. Int.*, **43**, 103–108.

Saishoji, A., Sato, D., Shishido, A. and Ikeda, T. (2007) 'Formation of bragg gratings with large angular multiplicity by means of the photoinduced reorientation of azobenzene copolymers', *Langmuir,* **23**, 320–326.

Shibaev, V., Bobrovsky, A. and Boiko, N. (2003) 'Photoactive liquid crystalline polymer systems with light-controllable structure and optical properties.', *Prog. Polym. Sci.,* **28**, 729–836.

Tang, X., Gao, L., Fan, X. and Zhou, Q. (2007) 'ABA-type amphiphilic triblock copolymers containing p-ethoxy azobenzene via atom transfer radical polymerization: Synthesis, characterization, and properties', *J. Polym. Sci. Polym. Chem.,* **45**, 2225.

Todorov T., Nikolova L. and Tomova, N. (1984) 'Polarization holography. 1: A new high-efficiency organic material with reversible photoinduced birefringence', *Appl.Opt.,* **23**, 4309–4312.

van Hest, J. C. M., Delnoye, D. A. P., Baars, M., Elissen-Roman, C., van Genderen, M. H. P. and Meijer, E. W. (1996) 'Polystyrene-poly(propylene imine) dendrimers: Synthesis, characterization, and association behavior of a new class of amphiphiles', *Chem. Eur. J.,* **2**(12), 1616–1626.

Wang, D., Ren, H., Wang, X. and Wang, X. (2008) 'Amphiphilic diblock copolymers functionalized with strong push–pull Azo chromophores: Synthesis and multimorphological aggregation', *Macromolecules,* **41**, 9382–9388.

Wang, D., Ye, G., Zhu, Y. and Wang, X. (2009) 'Photoinduced mass-migration behavior of two amphiphilic side-chain Azo diblock copolymers with different length flexible spacers', *Macromolecules,* **42**, 2651–2657.

Wang, X., Kumar, J., Tripathy, S. K., Li, L., Chen, J.-I. and Marturunkakul, S. (1997) 'Epoxy-based nonlinear optical polymers from post Azo coupling reaction', *Macromolecules,* **30**, 219–225.

Watabe, K. (2011) 'Next-generation optical disc technologies', *Toshiba Review,* **66**, 24–29.

Wurm, F. and Frey, J. (2011) 'Linear-dendritic block copolymers: The state of the art and exciting perspectives', *Prog. Polym. Sci.,* **36**, 1–52.

Xu, J., Zhang, W., Zhou, N., Zhu, J., Cheng, Z., Xu, Y. and Zhu, X. (2008) 'Synthesis of azobenzene-containing polymers via RAFT polymerization and investigation on intense fluorescence from aggregates of azobenzene-containing amphiphilic diblock copolymers', *J. Polym. Sci. Polym. Chem.,* **46**, 5652.

Yagai, S. and Kitamura, A. (2008) 'Recent advances in photoresponsive supramolecular self-assemblies', *Chem. Soc. Rev.*, **37**, 1520–1529.

Yoshida, T., Kanaoka, S. and Aoshima, S. (2005) 'Photosensitive copolymers with various types of azobenzene side groups synthesized by living cationic polymerization', *J. Polym. Sci. Polym. Chem.,* **43**, 4292–4297.

Yu, H. F., Asaoka, S., Shishido, A., Iyoda, T. and Ikeda, T. (2007) 'Photoinduced nanoscale cooperative motion in a well-defined triblock copolymer', *Small,* **3**, 768–771.

Yu, H., Naka, Y., Shishido, A. and Ikeda, T. (2008) 'Well-defined liquid-crystalline diblock copolymers with an azobenzene moiety: Synthesis, photoinduced alignment and their holographic properties', *Macromolecules,* **41**, 7959–7966.

Yu, H., Kobayashi, T. and Ge, Z. (2009) 'Precise control of photoinduced birefringence in azobenzene-containing liquid-crystalline polymers by post functionalization', *Macromol. Rapid Comm.,* **30**, 1725–1730.

Zegber, I., Rutloh, M., Hoffmann, U., Stumpe, J., Siesler, H. W. and Hvilsted, S. (2002) 'Photoorientation of a liquid crystalline polyester with azobenzene side groups. 1. Effects of irradiation with linearly polarized blue light', *J. Phys. Chem. A*, **106**, 3454–3462.

Zeng, Q., Li, Z. a., Li, Z., Ye, C., Qin, J. and Tang, B. Z. (2007) 'Convenient attachment of highly polar Azo chromophore moieties to disubstituted polyacetylene through polymer reactions by using "click" chemistry', *Macromolecules,* **40**, 5634–5637.

Zhang, Q., Bazuin, C. G. and Barrett, C. J. (2007a) 'Simple spacer-free dye-polyelectrolyte ionic complex: Side-chain liquid crystal order with high and stable photoinduced birefringence', *Chem. Mater.*, **20**, 29–31

Zhang, Y., Cheng, Z., Chen, X., Zhang, W., Wu, J., Zhu, J. and Zhu, X. (2007b) 'Synthesis and photoresponsive behaviors of well-defined azobenzene-containing polymers via RAFT polymerization', *Macromolecules,* **40**, 4809–4817.

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