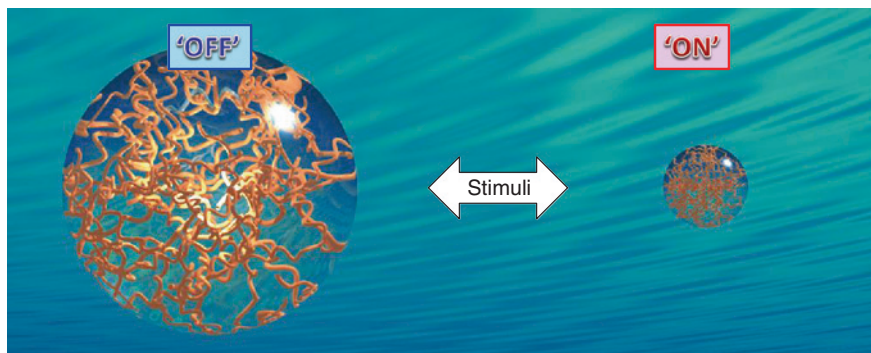


# Chapter 2

## Smart Hydrogels

### 2.1 Introduction

This chapter focuses on the synthesis, characterization, and applications of stimuli-responsive hydrogel-based materials. Hydrogels are three-dimensional (3D) materials with the ability to absorb large amounts of water while maintaining their dimensional stability. The 3D integrity of hydrogels in their swollen state is maintained by either physical or chemical crosslinking [1–3]. Chemically crosslinked networks have permanent junctions, while physical networks have transient junctions that arise from either polymer chain entanglements or physical interactions such as ionic interactions, hydrogen bonds, or hydrophobic interactions [4]. Indeed, there are many different macromolecular structures that are possible for physical and chemical hydrogels. They include the following: crosslinked or entangled networks of linear homopolymers, linear copolymers, and block or graft copolymers; polyion-multivalent ion, polyion–polyion or H-bonded complexes; hydrophilic networks stabilized by hydrophobic domains; and interpenetrating polymer networks (IPNs) or physical blends. Hydrogels may also have many different physical forms, including (a) solid molded forms (e.g., soft contact lenses), (b) pressed powder matrices (e.g., pills or capsules for oral ingestion), (c) microparticles (e.g., as bioadhesive carriers or wound treatments), (d) coatings (e.g., on implants or catheters; on pills or capsules, or coatings on the inside capillary wall in capillary electrophoresis), (e) membranes or sheets (e.g., as a reservoir in a transdermal drug delivery patch; or for 2D electrophoresis gels), (f) encapsulated solids (e.g., in osmotic pumps), and (g) liquids (e.g., that form gels upon heating or cooling) [5]. Hydrogels can also be separated into two groups on the basis of their natural or synthetic origins [6, 7]. Hydrogel-forming natural polymers include proteins such as collagen and gelatin, and polysaccharides such as alginate and agarose. These hydrogels have many advantageous features, including low toxicity and good biocompatibility, because their chemical structures are similar to those of the bioactive glycosaminoglycan (GAG) molecules (e.g., heparin sulfate, chondroitin sulfate, and hyaluronan) present in the native extracellular



**Fig. 2.1** Schematic illustration of a smart hydrogel that can undergo reversible volume phase transitions upon minute changes in environmental condition

matrix (ECM). Synthetic polymers that form hydrogels are traditionally prepared using chemical polymerization methods. Approaches applying genetic engineering and biosynthetic methods to create unique hydrogel materials have recently been reported [8, 9].

Hydrogels have been of great interest to biomaterial scientists for many years since the pioneering work of Wichterle and Lim in [10] on crosslinked 2-hydroxyethyl methacrylate (HEMA) hydrogels. Lower interfacial tension, soft and tissue like physical properties, higher permeability to undersized molecules, and release of entrapped molecules in a controlled manner have made hydrogels a focus of exploration in different biomedical fields. Successful examples include wound dressings [11–13], superabsorbents [14], drug delivery systems [15–18], and tissue engineering [17, 19]. In particular, hydrogels have been used extensively in the development of drug delivery systems, because hydrogels can not only protect the drug from hostile environments but also control drug release by changing the gel structure in response to environmental stimuli. Hydrogels containing such ‘sensor’ properties can undergo reversible volume phase transitions or gel–sol phase transitions upon minute changes in the environmental condition. These types of stimuli-responsive hydrogels are also called ‘smart’ hydrogels (Fig. 2.1) [20, 21]. Many physical and chemical stimuli have been applied to induce various responses of the smart hydrogel systems. The physical stimuli include temperature, electric fields, solvent composition, light, pressure, sound and magnetic fields, whereas the chemical or biochemical stimuli include pH, ions and specific molecular recognition events. Smart hydrogels have been used in diverse applications, such as in making actuators [22–25] and valves [26–28], in the immobilization of enzymes and cells [20, 29–31], in sensors [16, 32, 33], and in concentrating dilute solutions in bioseparation [34, 35].

Despite significant advances in smart hydrogels, however, conventional hydrogels have limited utility in manipulating their swelling/shrinking kinetics for practical applications owing to their size dependence [36]. As both gel swelling and

shrinking kinetics are typically governed by diffusion-limited polymer network transport in water, the inverse of the rate is proportional to the square of the gel dimension [37]. Therefore, the molecular design of polymer architectures of smart hydrogels is particularly important to show the potentially powerful combination of thermodynamic and kinetic regulation of smart hydrogels. Fast-response hydrogels, for example, benefit from converting external stimuli into local alteration of mechanical or physical properties that then prompt drug release and smart actuators. To increase the response of gel dynamics, several strategies have been explored. Owing to the intrinsic diffusion dependence, reducing gel size is one technique known to achieve rapid kinetics. Other techniques include making the gel heterogeneous, such as producing a microporous gel structure to increase the contacting surface area between polymer and solvent [38]. Novel strategies focusing on different hydrogel architectures have also been proposed [39–41].

This chapter focuses on smart hydrogels from the viewpoints of their preparation methods, characterizations and applications. Sections 2.2 and 2.3 describe the classifications of smart hydrogels on the basis of the preparation methods and stimuli, respectively. Special attention has been paid to the effects of hydrogel architecture on ‘on-off’ switchable swelling/shrinking properties, because the characteristics and some potential applications of the gels are related to their preparation methods. The characterization methods are discussed in Sect. 2.4. In Sect. 2.5, certain applications of the smart hydrogels are discussed. The chapter ends with a look at some of the future trends in the applications in biotechnology and biomedicine.

## 2.2 Classification on the Basis of Preparation Methods

Hydrogels can be classified in several ways depending on the preparation methods. Among them, one of the important classifications is based on their crosslinking nature. The detailed classification is presented in Table 2.1. In chemically crosslinked gels, covalent bonds are present between different polymer chains. Therefore, they are stable and cannot be dissolved in any solvents unless the covalent crosslink points are cleaved. In physically crosslinked gels, dissolution is prevented by physical interactions, which exist between different polymer chains. They are advantageous for a great number of pharmaceutical and biomedical applications because the use of crosslinking agents is avoided.

### 2.2.1 Physically Crosslinked Hydrogels

In recent years, there has been increasing interest in physically crosslinked gels. The main reason is that the use of crosslinking agents in the preparation of such hydrogels is avoided. To create physically crosslinked gels, different methods

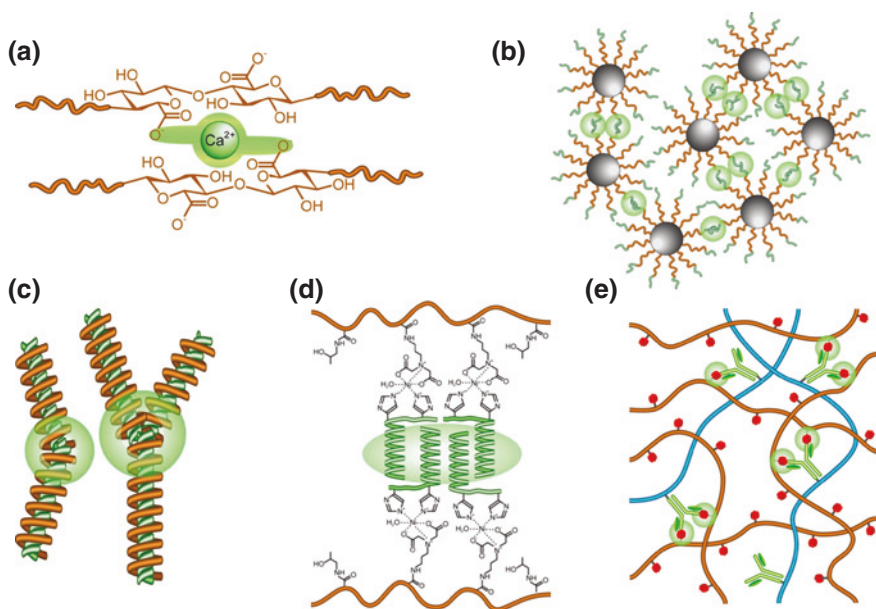
**Table 2.1** Methods for synthesizing physically and chemically crosslinked hydrogels

## Physically crosslinked hydrogels

- Ionic interactions (alginate etc.)
- Hydrophobic interactions (PEO–PPO–PEO etc.)
- Hydrogen bonding interactions (PAAc etc.)
- Stereocomplexation (enantiomeric lactic acid etc.)
- Supramolecular chemistry (inclusion complex etc.)

## Chemically crosslinked hydrogels

- Polymerization (acryloyl group etc.)
- Radiation ( $\gamma$ -ray etc.)
- Small-molecule crosslinking (glutaraldehyde etc.)
- Polymer–polymer crosslinking (condensation reaction etc.)



**Fig. 2.2** Schematic of methods for formation of physically crosslinked hydrogels via. **a** Ionic interactions, **b** hydrophobic interactions, **c** self-assembly of stereocomplex formation, **d** coiled-coil interactions, **e** specific molecular recognition

have been investigated. Alginate is a well-known example of a polymer that can be crosslinked by ionic interactions. It is a polysaccharide with mannuronic and glucuronic acid residues and can be crosslinked by calcium ions (Fig. 2.2a) [42]. Crosslinking can be carried out at room temperature and physiological pH. Therefore, alginate gels are frequently used as a matrix for the encapsulation of living cells [43] and for the release of proteins [44]. Interestingly, the gels can be destabilized by the extraction of Ca ions from the gel by a chelating agent. The

activity of incorporated proteins within the gel can be modulated by treating the particles with anionic polymers [45]. Iskakov et al. [46] have demonstrated time-programmed release of macromolecular drugs from calcium alginate gel beads modified with an anionic polymer, poly(carboxy-*n*-propylacrylamide-*co*-dimethylacrylamide) (P(CNPAAm-*co*-DMAAm), of varying coating thickness from 25 to 125  $\mu\text{m}$ . The lag time for pulsatile release of dextran was regulated by adjusting the copolymer coating thickness. The hydrolytic degradation of gel microspheres based on calcium crosslinked phosphazene polyelectrolytes, poly[di(carboxylatophenoxy)phosphazene] (PCPP) and poly[(carboxylatophenoxy)(glycinato)phosphazene] (PCGPP) was also demonstrated by Andrianov et al. [47]. The degradation rates can be increased by the incorporation of hydrolysis-sensitive glycinato groups as the pendant structures in the polymer. Alginate gel is also formed by gelation with polycations such as polylysine [48]. Ionic interaction is also formed by mixing negatively and positively charged microspheres. Dextran microspheres coated with anionic and cationic polymers exhibit spontaneous gelation upon mixing owing to ionic complex formation between the oppositely charged microparticles [49].

Hydrophobic interactions have also been exploited to design physically crosslinked gels. Amphiphilic block and graft copolymers can self-assemble in water to form organized structures such as polymeric micelles and hydrogels, in which the hydrophobic segments of the polymers are aggregated (Fig. 2.2b). Physically crosslinked hydrogels are generally obtained from multiblock copolymers or graft copolymers. The latter can be composed of a water-soluble polymer backbone, for example, a polysaccharide, to which hydrophobic units are attached, or hydrophobic chains containing water-soluble grafts. The most commonly used thermogelling polymers are Pluronics<sup>®</sup> and Tetronics<sup>®</sup> [50]. Micelles are formed at low concentrations in water, and at higher concentrations, thermo-reversible gels are formed. Some of them have been approved by the FDA and EPA for applications in food additives, pharmaceutical ingredients and agricultural products. To add a biodegradable capacity, the PPO segment of PEO-PPO-PEO block copolymers is often replaced by a biodegradable poly(L-lactic acid) (PLLA) [51] or poly(DL-lactic acid-*co*-glycolic acid) (PLGA) [52] segment. When low-molecular-weight PEG versus high-molecular-weight PLGA was used, the aqueous solution of PEG-PLGA-PEG triblock copolymer forms a solution at room temperature, where as at body temperature, it becomes a gel within a few seconds. The molecular architecture was not limited to the A-B-A-type block copolymer, but expanded into three-dimensional, hyper branched structures, such as a star-shaped structure [53]. Proper combinations of molecular weight and polymer architecture resulted in gels with different LCST values. Hydrophobic cholesterol-bearing pullulan also forms hydrogel nanoparticles upon self-aggregation in water [54, 55]. Chitosan solutions containing glycerol-2-phosphate ( $\beta$ -GP), which undergo temperature-controlled pH-dependent sol-gel transition at a temperature close to 37  $^{\circ}\text{C}$ , have recently been proposed [56, 57]. The combination of chitosan and PEG also forms a gel that releases bovine serum albumin (BSA) over 70 h [58]. Other chitosan hydrogels that respond to external changes have

been prepared by grafting with PNIPAAm [59]. This type of gel-forming polymer has recently become increasingly attractive as an injectable hydrogel for the development of therapeutic implants.

Hydrogen bonding interactions can also be used to form physically crosslinked gel-like structures. Mixtures of two or more natural polymers can display rheological synergism, meaning that the viscoelastic properties of the polymer blends are more gel-like than those of the constituent polymers measured individually [60–62]. Blends of, for example, gelatin–agar, starch–carboxymethyl cellulose, and hyaluronic acid–methylcellulose form physically crosslinked gel-like structures that are injectable. Poly(acrylic acid) (PAAc) and poly(methacrylic acid) (PMAAc) form complexes with PEG. These complexes are held together by hydrogen bonds between the oxygen of the PEG and the carboxylic group of PAAc or PMAAc, where hydrophobic interactions also play a role [63]. Hydrogen bonding has also been observed in poly(methacrylic acid-*g*-ethylene glycol) [P(MMc-*g*-EG)] [64]. The hydrogen bonds are only formed when the carboxylic acid groups are protonated. This implies that the swelling of these gels is strongly dependent on the pH. However, hydrogen-bonded networks can dilute and disperse over a few hours owing to the influx of water, restricting their use to relatively short-acting drug release systems [65].

Crystallization of polymers has also been used to form physically crosslinked gels. When aqueous solutions of poly(vinyl alcohol) (PVA) undergo a freeze–thawing process, a strong and highly elastic gel is formed. Gel formation is ascribed to the formation of PVA crystallites that act as physical crosslinking sites in the network [66]. Stenekes et al. [67] have reported the preparation of dextran hydrogels and microspheres based on crystallization. Although dextrans are known to be highly soluble in water, precipitation was observed in concentrated aqueous solutions of low-molecular-weight dextran. The precipitation process was accelerated by stirring and by the presence of salts. The precipitates were insoluble in water at room temperature, but readily dissolved in boiling water or dimethyl sulfoxide (DMSO).

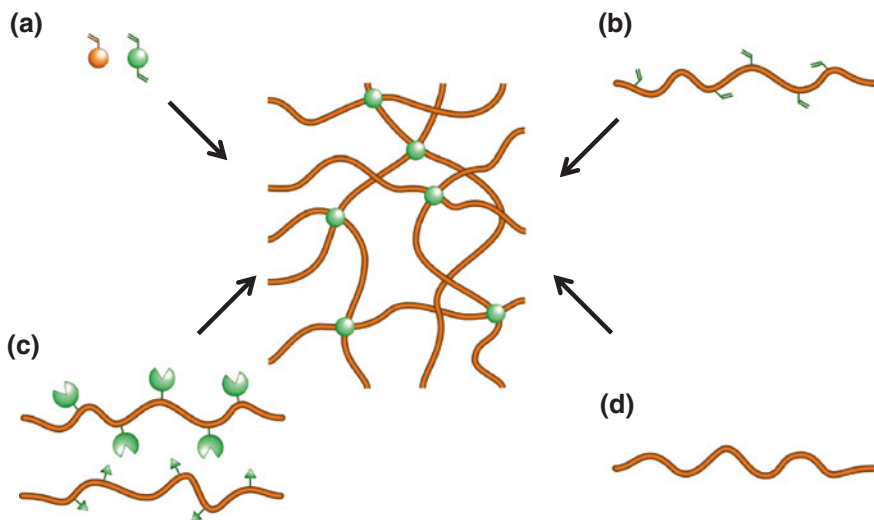
A novel hydrogel concept based on the self-assembly of a stereocomplex formation has been reported (Fig. 2.2c). The ability of PLA to form stereocomplexes was first described by Tsuji et al. [68]. In general, stereocomplex formation occurs in, for example, PLLA and PDLA. To create hydrogels crosslinked by stereocomplex formation, enantiomeric lactic acid oligomers were coupled to dextran [69]. In recent years, hydrogels have been described for drug delivery systems based on stereocomplex formation. In blends of triblock copolymers of PLLA–PEG–PLLA and PDLA–PEG–PDLA, stereocomplex formation occurs. The release of bovine serum albumin (BSA) from microspheres based on these triblock copolymers has been studied by Lim and Park [70]. The major advantage of this system is that a hydrogel was easily formed upon dissolving each product in water and mixing the solution. One significant limitation of stereocomplexation is, however, the relatively restricted range of polymer compositions that can be used.

The ubiquitous noncovalent interactions in biological systems are also being used to generate hydrogels with unique, dynamic functions [71]. Biological systems are dominated by noncovalent interactions, which can be defined as intermolecular



interactions, in which there is no change in either chemical bonding or electron pairing [72]. These interactions provide an excellent mechanism for dynamically regulating the assembly and function of biological systems. Petka et al. [8] have created hydrogels based on the “leucine zipper” motif. The formation of coiled-coil aggregates of the terminal domains in near-neutral aqueous solutions triggers the formation of a three-dimensional polymer network, with the polyelectrolyte segment retaining solvent and preventing precipitation of the chain. Dissociation of the coiled-coil aggregates through the elevation of pH or temperature causes dissolution of the gel and a return to the viscous behavior that is characteristic of polymer solutions. Another distinctive quality of proteins has been used to design proteins that self-assemble into fibers. In a particularly well-characterized example, repeating strands of  $\beta$ -sheet-forming peptides are used to drive the stacking assembly of amyloid-like nanofibers [73]. In addition, heterodimeric proteins with subunits that interact with one another via specific hetero-subunit interactions [74, 75] have been designed to assemble into two-dimensional protein filaments of less than 100 nm in diameter. Cappello et al. [76, 77] prepared sequential block copolymers containing a repetition of silk-like and elastine-like blocks, in which the insoluble silk-like segments are associated in the form of aligned hydrogen-bonded beta strands or sheets. Stewart et al. [9, 78] investigated natural and engineered proteins that show coiled-coil interactions and used the mas crosslinkers for poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA) (Fig. 2.2d). One end of the proteins was attached to the polymer backbone by metal complexes between histidine tags and metal-chelating ligands on the polymer. The hydrogel including the natural protein showed a temperature-induced collapse close to the melting temperature of the coiled-coil protein, which was attributed to the change from an elongated rod-like coiled-coil conformation to random coils. Thus, the large number of known protein–protein interactions and the now routine ability to synthesize new proteins or fusion proteins via recombinant DNA technology suggest that noncovalent assembly via protein–domain recognition could become an adaptable synthetic mechanism in bio-nanotechnology.

Gels can be formed by crosslinking interactions that occur upon antigen–antibody binding. Miyata et al. [79] prepared such a hydrogel by grafting the antigen and corresponding antibody to the polymer network, so that binding between the two introduces crosslinks in the network (Fig. 2.2e). Competitive binding of the free antigen triggers a change in gel volume owing to the breaking of these noncovalent crosslinks. Reversible swelling/shrinking was observed upon alternating exposure of the hydrogel to antigen-containing and antigen-free solutions. In addition, hydrogel membranes displaying on/off switching behavior with respect to protein permeation through the membranes were prepared, suggesting that this approach might permit drug delivery in response to a specific antigen. A highly specific interaction between glucose and Concanavalin A (Con A) has also been used to form crosslinks between glucose-containing polymer chains. Since Con A exists as a tetramer at physiological pH and each subunit has a glucose binding site, Con A can function as a crosslinking agent for glucose-containing polymer chains. Because of the noncovalent interaction between glucose and Con A, the formed crosslinks are reversible [80–82].



**Fig. 2.3** Schematic of methods for formation of chemically crosslinked hydrogels by radical polymerization of **a** vinyl monomers and **b** macromonomers **c** reaction of pendant functional groups, and **d** high-energy radiation

### 2.2.2 Chemically Crosslinked Hydrogels

While physically crosslinked hydrogels have the general advantages of forming gels without the need for chemical modification or the addition of crosslinking entities, they also have limitations. It is difficult to decouple variables such as gelation time, network pore size, chemical functionalization, and degradation time; this restricts the design flexibility of a physically crosslinked hydrogel because its strength is directly related to the chemical properties of the constituent gelators. In contrast, chemical crosslinking results in a network with a relatively high mechanical strength and, depending on the nature of the chemical bonds in the building blocks and the crosslinks, relatively long degradation times. Chemically crosslinked gels are also mechanically stable owing to the covalent bond in these gels. Chemically crosslinked gels can be obtained by radical polymerization of low-molecular-weight monomers in the presence of a crosslinking agent (Fig. 2.3a). One of the most widely used methods for the preparation of NIPAAm-based hydrogels is a redox polymerization using ammonium persulphate (APS) as an initiator and *N, N', N'-*tetramethylethylenediamine (TEMED) as a catalyst. TEMED accelerates the rate of formation of free radicals from persulfate, and these in turn catalyze polymerization. The persulfate free radicals convert monomers to free radicals that react with unactivated monomers to begin the polymerization chain reaction. The elongating polymer chains are randomly crosslinked by a crosslinker, resulting in a gel with a characteristic formulation that depends on such parameters as the polymerization conditions and monomer/



crosslinker concentrations. This is a very efficient system that results in the rapid formation of the gel even under mild conditions. Moreover, stimuli-responsive hydrogels can be easily obtained by copolymerization with, for example, NIPAAm or AAc. A great variety of smart hydrogels have been synthesized by this procedure [41, 79, 83–85]. However, unreacted peroxydisulfate and TEMED as well as their degradation products must be extracted from the gel before in vivo application. Moreover, this initiator system can also damage proteins once they are present during the preparation of the gels. In particular, methionine residues of the protein can be oxidized [86].

Aside from radical polymerization of mixtures of vinyl monomers, chemically crosslinked hydrogels can also be obtained by radical polymerization of polymers derivatized with polymerizable groups (macromonomer) (Fig. 2.3b). (Meth)acrylate groups can be introduced in water-soluble polymers using, for example (meth)acryloyl chloride, methacrylic anhydride, and bromoacetyl bromide. Moreover (meth)acrylic groups have been introduced in mono- and disaccharides, which can be used for the synthesis of hydrogels [87]. A hydrogel is formed after the addition of an APS/TEMED initiator system to an aqueous solution of the methacryl-dextran-containing *N, N'*-methylene-bis-acrylamide (MBAAm). Water-soluble polymers other than dextran, namely, albumin [88] (hydroxyethyl)starch [89], poly-aspartamide [90], poly(vinyl alcohol) [91] and hyaluronic acid [92], have also been derivatized with (meth)acrylic groups. In recent years, UV-induced polymerization has been frequently used to prepare hydrogels [93–96]. Moreover, with UV-induced polymerization, patterned structures can be prepared. It should be noted that when the UV polymerization is carried out in the presence of a drug, the network structure might be affected [97]. Moreover, the type of photo initiator as well as the solvent in which it is dissolved should be selected with care, since they may leak out from the formed hydrogel. Finally, once the polymerization is carried out in the presence of a protein, the potential damage of the radicals formed on the protein structure should be assessed [98].

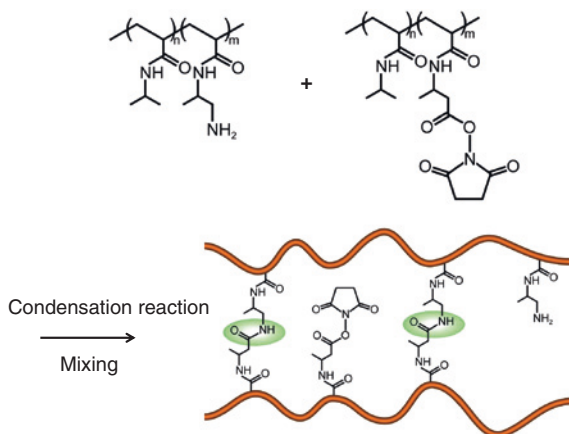
If polymers have pendant functional groups (e.g., OH, COOH, and NH<sub>2</sub>), covalent linkages between polymer chains can be established by the reaction of functional groups with complementary reactivity such as an amine-carboxylic acid or an isocyanate-OH/NH<sub>2</sub> reaction, or by Schiff base formation (Fig. 2.3c). For example, water-soluble polymers with hydroxyl groups can be crosslinked using glutaraldehyde [99, 100]. Amine-containing polymers can be crosslinked with the same reagent under mild conditions whereby so-called Schiff bases are formed. This has been investigated particularly for the preparation of crosslinked proteins [101, 102]. Because glutaraldehyde is a toxic compound that even at low concentration shows cell growth inhibition, alternatives have been developed. Crosslinking of gelatin using polyaldehydes obtained by partial oxidation of dextran has been reported [103]. Lee et al. [104] have reported crosslinking of poly(aldehyde guluronate) (PAG) with adipic acid dihydrazide. When an anti-neoplastic agent, daunomycin, was present during the hydrogel formation process, the drug was grafted onto the polymer matrix through a covalent linkage. Owing to the hydrolysis of this linkage, daunomycin was released in a time frame

of 2 days to 6 weeks [105]. Hyaluronic acid hydrogels were also obtained by the derivatization of hyaluronic acid with adipic dihydrazide followed by crosslinking with a macromolecular crosslinker. These gels are enzymatically degradable with hyaluronidase and therefore have the potential to act as a delivery matrix for sustained release of drugs at wound sites [106]. Water-soluble polymers can also be converted into hydrogels via addition reactions. For example, polysaccharides can be crosslinked with 1, 6-hexamethylenediisocyanate [107], divinyl sulfone [108], or 1, 6-hexane dibromide [109] and many other reagents. The network properties can be easily tailored by adjusting the concentration of the dissolved polymer and the amount of crosslinking agent. However, the crosslinking reactions are preferably carried out in organic solvents, because water can also react with the crosslinking agent. Furthermore, since the crosslinking agents are toxic, the gels must be extracted extensively to remove traces of unreacted agents.

Condensation reactions between hydroxyl groups or amines with carboxylic acids or derivatives are frequently applied to the synthesis of polymers to yield polyesters and polyamides, respectively. These reactions can also be used for the preparation of hydrogels. A very efficient reagent to crosslink water-soluble polymers with amide bonds is *N,N*-(3-dimethylaminopropyl)-*N*-ethyl carbodiimide (EDC). Kuijpers et al. [110] described the preparation of gelatin hydrogels using this reagent. During the reaction, *N*-hydroxysuccinimide (NHS) is added to suppress possible side reactions and to have better control over the crosslink density of the gels. Eiselt et al. [111] developed a method to covalently crosslink alginate and PEG-diamines using EDC in order to obtain alginate gels with better mechanical properties than the ionically crosslinked gels. The mechanical properties could be controlled by adjusting the amount of PEG-diamine in the gel and the molecular weight of PEG. de Nooy et al. [112, 113] have described the synthesis of polysaccharide hydrogels via the Passerini and Ugi condensation reactions. In the Passerini condensation, a carboxylic acid and an aldehyde or ketone are condensed with an isocyanide to yield an  $\alpha$ -(acryloxy)amide. In the Ugi condensation, an amine is added to this reaction mixture, finally yielding an  $\alpha$ -(acylamino)amide. The reaction can be carried out in water at slightly acidic pH and at room temperature. Since the Passerini condensation yields hydrogels with ester bonds in their crosslinks, these gels degrade at ambient temperature and pH 9.5. Since gels prepared using the Ugi condensation contain amide bonds in their crosslinks, these gels were stable under these conditions. Yoshida et al. [114] have prepared temperature-responsive hydrogels using NIPAAm copolymers with poly(amino acid)s as a side-chain group and activated ester groups. The hydrogels easily crosslinked with the degradable poly(amino acid) chains upon merely mixing the copolymer aqueous solutions (Fig. 2.4).

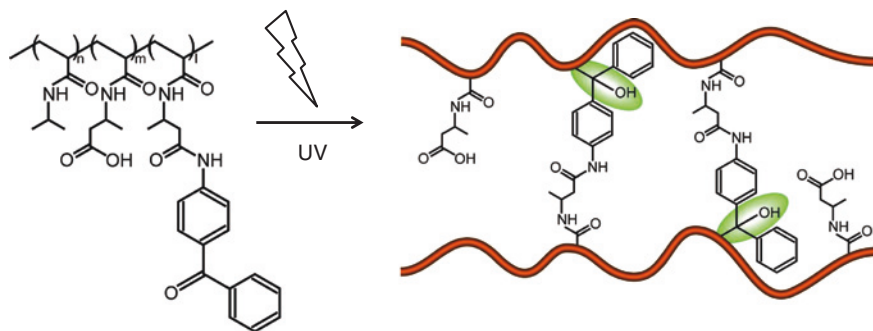
A novel hydrogel concept based on enzymatic reaction has also been reported. A tetrahydroxy PEG was functionalized with glutaminyl groups (PEG-Qa). PEG networks were then formed by the addition of transglutaminase to aqueous solutions of PEG-Qa and poly(lysine-*co*-phenylalanine) [115]. This enzyme catalyzes the reaction between the  $\gamma$ -carboxamide group of the PEG-Qa and the  $\epsilon$ -amine group of lysine to yield an amide linkage between the polymers. Poly(lysine-*co*-phenylalanine) was also replaced by lysine end-functionalized PEG, and

**Fig. 2.4** Preparation of a temperature-responsive hydrogel crosslinked with biodegradable poly(amino acid) chains via condensation reaction [114]



hydrogels were formed once transglutaminase was added to an aqueous solution of peptide-modified macromers [116].

High-energy radiation, such as gamma ( $\gamma$ ) and electron beam radiation, can be used to polymerize unsaturated compounds (Fig. 2.3d). On exposure to  $\gamma$  or electron beam radiation, aqueous solutions of polymers form radicals on the polymer chains (e.g., by the homolytic scission of C–H bonds). Also, the radiolysis of water molecules generates the formation of hydroxyl groups that can attack polymer chains, again resulting in the formation of microradicals. Recombination of these microradicals on different chains results in the formation of covalent bonds and finally in a crosslinked structure. PVA [117], PEG [118], and PAAc [119] are well-known examples of polymers that can be crosslinked with high-energy irradiation. The swelling and permeability characteristics of the gel depend on the extent of polymerization as a function of polymer and radiation dose (in general, crosslinking density increases with increasing radiation dose). Hirasa et al. [120, 121] have reported on fast-response, temperature-sensitive poly(vinyl methyl ether) (PVME) and PNIPAAm hydrogels prepared by  $\gamma$ -ray irradiation [122]. The structure of PVME hydrogels is dependent on the intensity of the  $\gamma$ -rays and the temperature during irradiation. When the radiation intensity is lower than  $1.5 \text{ kGy h}^{-1}$ , the temperature of the PVME solution does not change at room temperature during irradiation. Under this condition, PVME was crosslinked below LCST; therefore, a transparent gel with a homogeneous and dense structure was formed. On the other hand, the temperature of the PVME solution was increased by exposure to radiation of high intensity ( $9.8 \text{ kGy h}^{-1}$ ). At this position, an opaque gel with a heterogeneous and microporous structure was formed. This gel had a large surface area for its volume, and swelled and shrank very quickly upon changing the temperature. The author's group has designed photo-crosslinkable NIPAAm copolymers with a UV-reactive benzophenone (BP) conjugated comonomer [31, 123]. The photo-crosslinking was carried out by making use of the photo chemistry of the BP groups, the photochemically produced triplet state of which can abstract hydrogen atoms from almost any polymer, thus generating radicals (Fig. 2.5). In general, BP is excited indirectly to the lowest triplet state ( $\pi$ – $\pi^*$ ) by direct absorption into the



**Fig. 2.5** Photo-crosslinking of UV-reactive benzophenone conjugated temperature-responsive copolymer [31, 123]

singlet state ( $\pi-\pi^*$ ) upon irradiation with UV light. The BP ketyl radical and an on-chain polymer radical readily recombine to generate a new C–C bond, thereby resulting in crosslinking within the polymer networks. The advantage of using this process for gel formation is that it can be carried out in water under mild conditions without the use of a crosslinking agent. However, there are some drawbacks to using this method; the bioactive material must be loaded after gel formation, as irradiation might damage the agent. Also, in some gels such as PEG and PVA, the crosslinks consist of C–C bonds, which are not biodegradable.

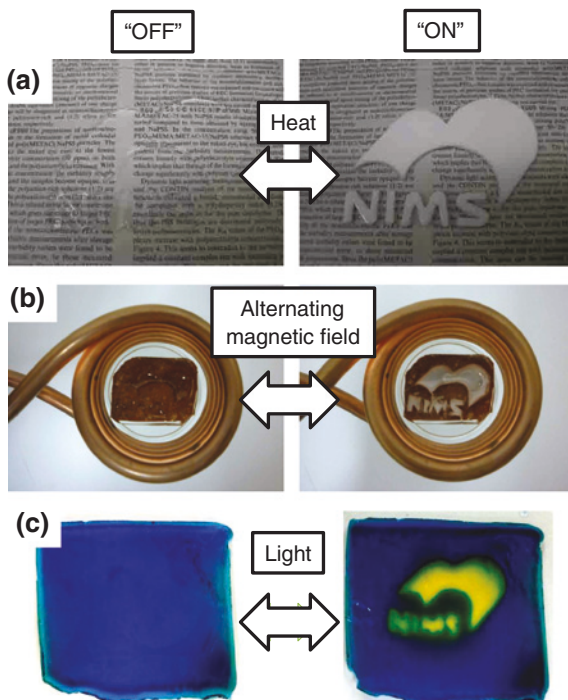
## 2.3 Classification on the Basis of Stimuli

Smart hydrogels could be further classified as either physical- or chemical-stimuli-responsive hydrogels. The physical stimuli, such as temperature, electric or magnetic fields, and mechanical stress, will affect the level of various energy sources and alter molecular interactions at critical onset points (Fig. 2.6). Chemical stimuli, such as pH, ionic factors and chemical agents, will change the interactions between polymer chains or between polymer chains and solvent at the molecular level. Recently, biochemical stimuli have been considered as another category that involves the responses to antigen, enzyme, ligand, and other biochemical agents. Some systems have been developed to combine two stimuli-responsive mechanisms into one polymer system, the so-called dual-responsive polymer systems.

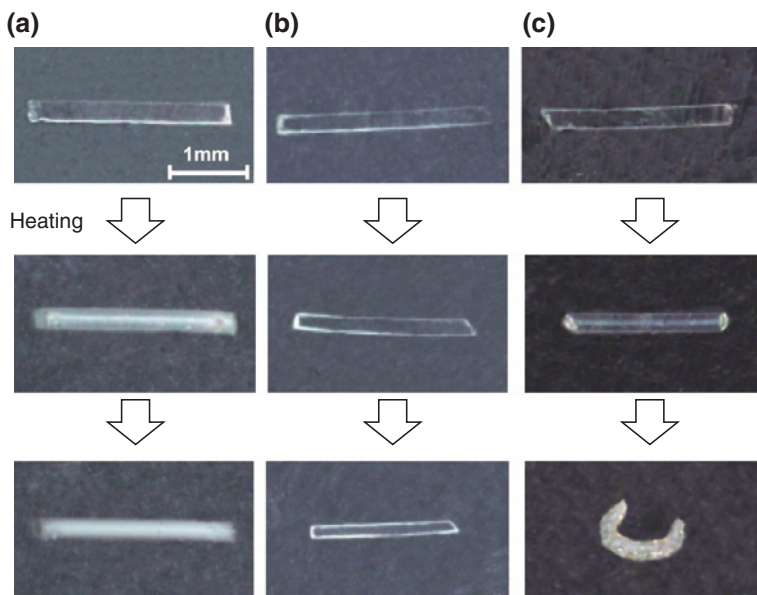
### 2.3.1 Physical Stimuli

Temperature-responsive hydrogels are probably the most commonly studied class of environmentally sensitive polymer systems. Temperature-responsive hydrogels can be classified as positive or negative temperature-responsive systems.

**Fig. 2.6** Photographs of **a** temperature-, **b** alternating-magnetic-field-, and **c** photoresponsive hydrogels



Physically crosslinked thermosensitive hydrogels may undergo sol–gel phase transitions instead of volume change at a critical solution temperature. Positive temperature-responsive hydrogels show phase transition at a critical temperature called the upper critical solution temperature (UCST). Hydrogels made from polymers with UCST shrink when cooled below their UCST. Negative temperature-responsive hydrogels have a lower critical solution temperature (LCST). These hydrogels shrink upon heating at above their LCST (see [Chap. 1](#)). Chemically crosslinked thermosensitive hydrogels undergo volume change rather than sol–gel transitions. Certain molecular interactions, such as hydrophobic associations and hydrogen bonds, play a vital role in the abrupt volume change of these hydrogels at the critical solution temperature. In the swollen state, water molecules form hydrogen bonds with polar groups of polymer backbone within the hydrogels and organize around hydrophobic groups as iceberg water. At the critical solution temperature, hydrogen bonding between the polymer and water, compared with polymer–polymer and water–water interactions, becomes unfavorable. This forces the quick dehydration of the system and water is released out of the hydrogel with a large gain in entropy, resulting in shrinkage of the polymeric structure. Of the many temperature-responsive polymers, PNIPAAm is probably the most extensively used because its LCST is close to body temperature. Copolymers of NIPAAm can also be made using other monomers to alter the LCST.



**Fig. 2.7** Photographs of shrinking behaviors of **a** PNIPAAm, **b** P(NIPAAm-*co*-AAc), and **c** P(NIPAAm-*co*-CIPAAm) hydrogels after temperature change from 10 to 40 °C [128]

In general, the sudden temperature changes from below to above the transition temperature lead to the formation of dense and less permeable surface skin layers on PNIPAAm gels [124]. The PNIPAAm gel changes from transparent to opaque after temperature change (Fig. 2.7a). To accelerate gel shrinkage, the introduction of hydrophilic molecules such as AAc into gels is one promising strategy (Fig. 2.7b). However, random introduction of a large amount of hydrophilic monomers into PNIPAAm hydrogels without compromising their intrinsic temperature sensitivity has proven difficult [125]. To overcome this, the authors have synthesized a new 2-carboxyisopropylacrylamide (CIPAAm) with structural similarity to NIPAAm side chains but also including a carboxylate group [126]. NIPAAm-CIPAAm hydrogels exhibited large and sensitive volume phase transitions in response to temperature changes even though carboxylate groups in CIPAAm exist in dissociated states at pH 7.4 [40, 127]. Initially transparent gels turned opaque upon heating from 10 to 40 °C owing to skin layer formation at the gel surface (Fig. 2.7c). However, gel shrinking was not stopped, regardless of the skin layer formation, owing to the sufficient hydrophilic carboxylate content allowing water movement [128]. Therefore, maintaining the isopropylamide side chain alignment within the copolymer chains may facilitate the introduction of large amounts of functional groups into NIPAAm copolymer gels without losing phase transition behavior. The new monomer, CIPAAm, should prove useful in introducing functional carboxyl groups into temperature-responsive PNIPAAm hydrogels while



maintaining their intrinsic temperature-sensitive behavior. Certain types of block copolymers made of poly(ethylene oxide)(PEO) and poly(propylene oxide) (PPO) also possess an inverse temperature-sensitive property. Because of their LCST at around body temperature, they have been used widely in the development of controlled drug delivery systems based on the sol–gel phase conversion at body temperature. A large number of PEO–PPO block copolymers are commercially available under the names of Pluronic® (or Poloxamers®) and Tetronics® [50]. Temperature-sensitive hydrogels can also be made using temperature-sensitive crosslinking agents. A hybrid hydrogel system was assembled from water-soluble synthetic polymers and a well-defined protein-folding motif, the coiled coil [9]. The hydrogel under went temperature-induced collapse owing to the cooperative conformational transition. Using temperature-sensitive crosslinking agents adds a new dimension in designing temperature-sensitive hydrogels.

Photoresponsive hydrogels have been used in a number of biotechnology applications, such as photocontrolled enzymatic bioprocessing [129], phototriggered targeted drug delivery systems [130], and photocontrolled separation/recovery systems in bioMEMs formats. Since the light stimulus can be imposed instantly and delivered in specific amounts with high accuracy, light-sensitive hydrogels may possess special advantages over others [131]. For example, the sensitivity of temperature-sensitive hydrogels is rate limited by thermal diffusion, while pH-sensitive hydrogels can be limited by hydrogen ion diffusion. The capacity for instantaneous delivery of the sol–gel stimulus makes the development of light-sensitive hydrogels important for various applications in both engineering and biochemical fields. Photoresponsive hydrogels can be separated into UV-sensitive and visible-light-sensitive hydrogels. Unlike UV light, visible light is readily available, inexpensive, safe, clean and easily manipulated. Typical photoreactive guests in polymers are azobenzene [132], triphenylmethane [133] and spiropyran [134] groups, which have been entrapped, crosslinked, and introduced as side chains or part of the main chain in polymer matrices. The UV-sensitive hydrogels were synthesized by introducing a leuco derivative molecule, bis(4-dimethylamino)phenylmethylleucocyanide, into the polymer network [133]. Triphenylmethane leuco derivatives are normally neutral but dissociate into ion pairs under ultraviolet irradiation, producing triphenylmethyl cations. Because the leuco derivative molecule can be ionized upon ultraviolet irradiation, the UV-light-induced swelling was observed owing to an increase in osmotic pressure within the gel. Sumaru et al. [134] have prepared a photo responsive hydrogel by radical copolymerization of NIPAAm, a vinyl monomer having a spirobenzopyran residue, and a crosslinker. It was observed that the permeability for a 1 mM HCl aqueous solution increased twofold in response to the blue light irradiation, and this photo response of the permeability was confirmed to be repeatable. Takashima et al. [135] have designed a photo responsive supramolecular actuator by integrating host–guest interactions and photoswitching ability in a hydrogel. A photoresponsive supramolecular hydrogel with  $\alpha$ -cyclodextrin as a host molecule and an azobenzene derivative as a photoresponsive guest molecule exhibits reversible macroscopic deformations

in both size and shape when irradiated by ultraviolet light at 365 nm or visible light at 430 nm. The deformation of the supramolecular hydrogel depends on the incident direction. The selectivity of the incident direction allows plate-shaped hydrogels to bend in water. Irradiating with visible light immediately restores the deformed hydrogel. A light-driven supramolecular actuator with  $\alpha$ -cyclodextrin and azobenzene stems from the formation and dissociation of an inclusion complex by ultraviolet or visible light irradiation. Visible-light-sensitive hydrogels can also be prepared by introducing a light-sensitive chromophore (e.g., trisodium salt of copper chlorophyllin) to NIPAAm hydrogels [136]. When light (e.g., 488 nm) is applied to the hydrogel, it is absorbed by the chromophore, and then dissipated locally as heat by radiationless transitions, increasing the 'local' temperature of the hydrogel. The temperature increase alters the swelling behavior of NIPAAm hydrogels. The authors have integrated a photoinitiated proton-releasing reaction of *o*-nitrobenzaldehyde (NBA) into pH-responsive hydrogels [137]. NBA-integrated hydrogels demonstrated quick release of protons upon UV irradiation, allowing the pH inside the gel to decrease below the  $pK_a$  of the polymer within one minute. Spatial control of gel shrinkage was also made possible by irradiating UV light to a limited region of the gel through a photomask.

Electric current can also be used as an environmental signal to induce responses of hydrogels. Hydrogels sensitive to electric current are usually made of polyelectrolytes, as are pH-sensitive hydrogels. Electroresponsive hydrogels undergo shrinking or swelling in the presence of an applied electric field. Sometimes, the hydrogels show swelling on one side and shrinking on the other side, resulting in the bending of the hydrogels. Osada and Hasebe [138] reported an electrically activated artificial muscle system that is contracted by an electrical stimulus under isothermal conditions. They reported that the addition of NaCl increased the rate of water release, whereas the addition of organic solvents such as ethanol, acetone, or water reduced the rate of water release, and the contraction that resulted from the electrostatic interaction between charged macromolecules and the electrodes led to extensive dehydration of the gel. Tanaka et al. [139] studied the effect of electric current on the contraction behavior of partially hydrolyzed polyacrylamide gels in a mixture of 50 % acetone and water. They observed that the contraction was most significant and rapid in water, whereas with increasing acetone percentage, the rate of contraction decreased gradually.

The concept that hydrogels may undergo pressure-induced volume phase transition originates from thermodynamic calculations based on the uncharged hydrogel theory. According to the theory, hydrogels that are collapsed at low pressure would expand at higher pressure. Experiments with PNIPAAm hydrogels confirmed this prediction [140]. The effect of hydrostatic pressure on the swelling of temperature-sensitive gels has also been studied by measuring the volume change of the beads of PNIPAAm gel, poly(*N*-*n*-propylacrylamide) gel, and poly(*N*, *N*-diethylacrylamide) gel under pressure up to 120 atm [141]. The excess enthalpy and excess volume of the gel-water systems during the volume phase transition of the gels were measured. The degree of swelling of hydrogels increased under hydrostatic pressure when the temperature was close to its LCST.

### 2.3.2 Chemical Stimuli

While physical stimuli are advantageous because they allow local and remote control, they result in a discontinuous response when the stimulus is turned 'off'. In other words, only the illuminated region is active, and continuous illumination is necessary. In the human body, however, the appearance of numerous bioactive molecules is tightly controlled to maintain a normal metabolic balance via the feedback system called homeostasis. For example, hormones or cytokines not only act locally (local signals), but also travel to other locations in the body via blood circulation (remote signals) [142]. These signals are sometimes amplified or transferred to another signal by sequentially interacting with many other different molecules. A representative process is blood coagulation, which is a complex sequence involving numerous clotting factors. The concentration gradients of protons (pH), ions, and oxidizing or reducing agents are also important characteristics observed in living systems. The human body exhibits variations in pH along the gastrointestinal tract, tumoral areas, inflamed or infected tissues, and the endosomal lumen.

pH-responsive hydrogels are made of polymeric backbones with ionic pendant groups that can accept or donate protons in response to an environmental pH change. As the environmental pH changes, the degree of ionization in a pH-responsive hydrogel is dramatically changed at a specific pH known as  $pK_a$  or  $pK_b$ . This rapid change in the net charge of ionized pendant groups causes abrupt volume transition by generating electrostatic repulsive forces between ionized groups, which creates a large osmotic swelling force. There are two types of pH-responsive hydrogels: anionic and cationic hydrogels. Poly(acrylic acid) (PAAc) becomes ionized at high pH, whereas poly(*N, N'*-diethylaminoethyl methacrylate) (PDEAEM) becomes ionized at low pH. pH-sensitive hydrogels have been most frequently used to develop controlled release formulations for oral administration. The pH in the stomach (<3) is quite different from the neutral pH in the intestine, and such a difference is large enough to elicit pH-dependent behavior of polyelectrolyte hydrogels. Hydrogels made of PAAc or PMAAc can be used to develop formulations that release drugs in a neutral pH environment [143]. Hydrogels made of polyanions (e.g., PAA) were developed for colon-specific drug delivery. Swelling of such hydrogels in the stomach is minimal, and thus, the drug release is also minimal. The extent of swelling increases as the hydrogel passes down the intestinal tract owing to the increase in pH leading to the ionization of the carboxylic groups. However, only in the colon can the azoaromatic crosslinks of the hydrogels be degraded by azoreductase produced by the microbial flora of the colon [144]. On the other hand, when a drug is loaded into hydrogels made of copolymers of MMA and *N, N'*-dimethylaminoethyl methacrylate (DMAEM), it is released at zero order at pH 3–5, but not released at neutral pH [145]. The lower extracellular pH of solid tumors has also been exploited in many therapeutic strategies based on drug delivery [146]. These extra cellular microenvironments have an acidic pH primarily because of the accumulation of excess lactic acid, which

is produced because of the high rate of glycolysis in tumor microenvironments [147]. Some other pathologic tissues, such as ischemic or infected sites, are also more acidic than normal tissues. In addition, the pH values of endosomal and lysosomal vesicles inside cells are lower than that of the cytosol, and this difference has been utilized for intracellular delivery [148]. Garbern et al. [149] have reported the use of pH- and temperature-responsive injectable hydrogels, synthesized from copolymers of NIPAAm and propylacrylic acid (PAAc), for delivering drugs to regions of local acidosis. P(NIPAAm-co-PAA) exhibits a sharp transition near body temperature, as indicated by its LCST in the pH range of 5–6. This system undergoes reversible gelation at moderately acidic pH values ( $\sim$ pH 5–6), but remains soluble at normal physiological pH (7.4). In general, the incorporation of carboxylic acid-derived monomers, such as AAC or MAAC, results in low  $pK_a$  values, which limit the use of these polymers for drug targeting to very low pH systems, such as the stomach. Because PAA polymers can also destabilize membranes in the endosome, this pH-responsive system has been shown to enhance the cytosolic delivery of nucleic acids [150], anticancer drugs [151], and an internalizing antibody [152].

Glucose-sensitive hydrogels have also been developed by many researchers because one of the most challenging problems in controlled drug delivery is the development of self-regulated (modulated) insulin delivery systems. The delivery of insulin is different from the delivery of other drugs, since insulin must be delivered in an exact amount at the exact time of need. Thus, self-regulated insulin delivery systems require glucose-sensing ability and an automatic shut-off mechanism. Many hydrogel systems have been developed for modulating insulin delivery, and all of them have a glucose sensor built into the system. Con A has been frequently used in modulated insulin delivery [82]. In this type of system, insulin molecules are attached to a support or carrier through specific interactions that can be interrupted by glucose itself. This generally requires the introduction of functional groups onto insulin molecules. In one approach, insulin was chemically modified to introduce glucose, which binds particularly to Con A [153]. Glucose-sensitive phase-reversible hydrogels can also be prepared without using Con A. Kataoka and coworkers have developed a self-regulated insulin delivery system using phenylboronic acid (PBA), a synthetic molecule capable of reversibly binding with 1, 2- or 1, 3-cis-diols including glucose [16, 154–156]. PBA and its derivatives are known to form covalent complexes with polyol compounds including glucose (Fig. 2.8). The glucose-dependent shift in the equilibrium of PBA between the uncharged and anionically charged forms, when coupled with a properly amphiphilic three-dimensional backbone (or gel), could induce a reversible change in the volume of the gel. The resultant rapid change in hydration under certain conditions could cause a localized dehydration of the gel surface, that is, the so-called skin layer, thus offering a method for instantly controlling the permeation of gel-loaded insulin.

For some biomedical applications, it is highly desirable and useful to develop a material or device that can respond to specific proteins such as antigens [157]. The concept is the same as that used in glucose-sensitive phase-reversible hydrogels. A

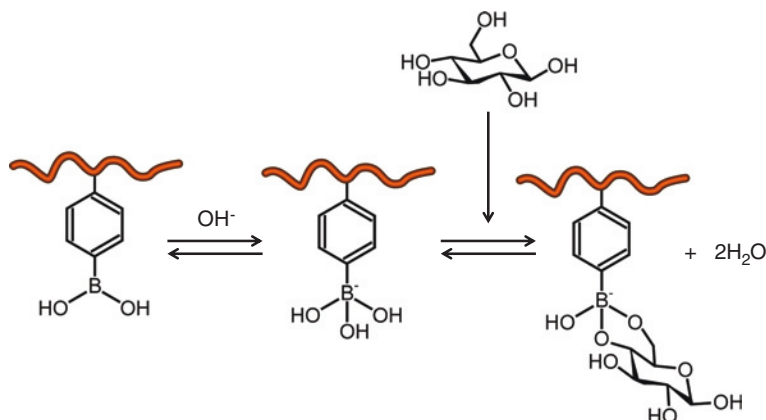


Fig. 2.8 Glucose-dependent equilibria of phenylboronic acid [16, 154–156]

semi-interpenetrating network hydrogel was prepared by grafting an antigen and a corresponding antibody to different polymer networks [79]. The gel is formed by crosslinking interactions that occur upon antigen–antibody binding. Hydrogel swelling is triggered in the presence of free antigens that compete with the polymer-bound antigen, leading to a reduction in the crosslinking density. Suzuki et al. [158] reported thrombin-induced infection-responsive hydrogels for the controlled release of antibiotics at the site and time of infection. PVA hydrogels loaded with grafted gentamycin were prepared. Gentamycin was chemically attached to the polymer backbone through peptide linkers that can be enzymatically degraded by thrombin. Exudates from the dorsal pouch of rats infected by *Pseudomonas aeruginosa* showed significantly higher thrombin like enzymatic activity toward a certain peptide sequence than exudates from noninfected wounds. DNA-responsive hydrogels have also been reported to be capable of swelling and shrinking in response to specific DNAs [159, 160].

Yoshida and coworkers have demonstrated an autonomic swelling-shrinking oscillation by integrating the chemical oscillation of the Belousov–Zhabotinsky (BZ) reaction into the hydrogel [161–163]. The BZ reaction is often analogically compared with the TCA cycle, which is a key metabolic process taking place in the living body. The overall process of the BZ reaction is the oxidation of an organic substrate, such as malonic acid (MA) or citric acid, by an oxidizing agent (bromate ion) in the presence of a strong acid and a metal catalyst. A copolymer gel that consists of NIPAAm and ruthenium tris(2, 2′-bipyridine) ( $\text{Ru}(\text{bpy})_3^{2+}$ ) was prepared. The  $\text{Ru}(\text{bpy})_3^{2+}$ , acting as a catalyst for the BZ reaction, was appended to the polymer chains of NIPAAm. The poly(NIPAAm-co- $\text{Ru}(\text{bpy})_3^{2+}$ ) gels have a phase transition temperature owing to the thermosensitive constituent NIPAAm. They have further demonstrated a novel biomimetic ‘self-walking’ gel actuator that can walk spontaneously with a worm like motion without switching of external stimuli [23].

## 2.4 Characterization Methods

A variety of techniques for characterizing hydrogels have been reported in the literature. The physical behavior of hydrogels is dependent on their equilibrium and dynamic swelling behavior in water, since upon preparation, they must be brought into contact with water to yield the final, swollen network structure. The most important parameters that define the structure and properties of swollen hydrogels are the polymer volume fraction in the swollen state, the effective molecular weight of the polymer chain between crosslinking points, and the correlation distance between two adjacent crosslinks [164, 165]. Rubber-elasticity theory and equilibrium-swelling theory are extensively applied to describe these three dependent parameters. The theoretical basis for the understanding of polymer solutions was developed independently by Flory [166] and Huggins [167] 70 years ago. Hydrogels have a variety of properties, such as absorption capacity, swelling behavior, permeability, surface properties, optical properties and mechanical properties, which make them promising materials for a wide variety of applications. The characteristics of the polymer chains and the crosslinking structures in these aqueous solutions play an important role in the outcome of the properties of the hydrogel.

### 2.4.1 Water in Hydrogels

When a dry hydrogel begins to absorb water, the first water molecules entering the matrix will hydrate the most polar, hydrophilic groups, leading to 'primary bound water'. As the polar groups are hydrated, the network swells and exposes hydrophobic groups, which also interact with water molecules, leading to hydrophobically bound water or 'secondary bound water'. Primary and secondary bound water are often combined and simply called 'total bound water'. After the water has interacted with both hydrophilic and hydrophobic sites, the osmotic driving force of the network chains allows the network to absorb more water. Finally, the balance between the retraction force and the infinite dilution force establishes an equilibrium swelling level. The additional water absorbed beyond the total bound water is defined as 'free water' or 'bulk water' [21]. There are a number of methods for estimating the relative amounts of free and bound water, as fractions of the total water content. The use of small molecular probes, DSC, and NMR are the three major methods. When probe molecules are used, the labeled probe solution is equilibrated with the hydrogel, and the concentration of the probe molecule in the gel at equilibrium is measured. The use of DSC is based on the assumption that only the free water may be frozen, so it is assumed that the endotherm measured when warming the frozen gel represents the melting of the free water, and that value will yield the amount of free water in the hydrogel sample being tested.



### 2.4.2 Thermodynamics of Hydrogel Swelling

The physical behavior of hydrogels is dependent on their equilibrium and dynamic swelling behavior in water. The Flory-Huggins theory can be used to calculate the thermodynamic behavior of hydrogel swelling [166, 167]. Considering the isotropic crosslinked structure of hydrogel, the total Gibbs free energy change of the system, upon swelling, can be written as

$$\Delta G = \Delta G_{\text{mixture}} + \Delta G_{\text{elastic}}. \quad (2.1)$$

Here,  $\Delta G_{\text{elastic}}$  is the contribution due to the elastic retractive forces and  $\Delta G_{\text{mixture}}$  represents the thermodynamic compatibility of the polymer and the swelling agent (water).

In order to express the chemical potential change of water in terms of elastic and mixing contributions at any time of swelling, the differentiating Eq. (2.1) with respect to the water molecules in the system gives

$$\mu_1 - \mu_{1,0} = \Delta\mu_{\text{mixture}} + \Delta\mu_{\text{elastic}}. \quad (2.2)$$

Here,  $\mu_1$  is the chemical potential of water within the gel and  $\mu_{1,0}$  is the chemical potential of pure water.

At equilibrium, the chemical potentials of water inside and outside of the gel must be equal. Therefore, the elastic and mixing contributions to the chemical potential will balance one another at equilibrium. The change in chemical potential upon mixing can be determined from the heat of mixing and the entropy of mixing. Using the Flory-Huggins theory, the chemical potential of mixing can be expressed as

$$\Delta\mu_{\text{mixture}} = RT(\ln(1 - 2v_{2,s}) + v_{2,s} + \chi_1 v_{(2,s)}^2), \quad (2.3)$$

where  $\chi_1$  is the polymer-water interaction parameter,  $v_{2,s}$  is the polymer volume fraction of the gel,  $T$  is the absolute temperature, and  $R$  is the gas constant.

This thermodynamic swelling contribution is counter balanced by the retractive elastic contribution of the crosslinked structure. The latter is usually described by the rubber elasticity theory and its variations. Equilibrium is attained in a particular solvent at a particular temperature when the two forces become equal. The volume degree of swelling,  $Q$  (i.e., the ratio of the actual volume of a sample in the swollen state divided by its volume in the dry state), can then be determined from Eq. (2.4).

$$v_{2,s} = V_p/V_{\text{gel}} = 1/Q \quad (2.4)$$

### 2.4.3 Swelling Ratios

The swelling behavior of hydrogel systems is an important parameter governing their applications specifically in pharmaceutical, ophthalmology and tissue engineering. The polymer chains in a hydrogel interact with the solvent molecule and tend to expand to the fully solvated state, while the crosslinked structure applies

a retractive force to pull the chains inside. Equilibrium is achieved when these expanding and retracting forces counter balance each other. The equilibrium swelling ratio or water content, given by Eq. (2.5), is generally used to describe the swelling behavior of hydrogels.

$$\text{Equilibrium swelling ratio} = W_{\text{swollen}}/W_{\text{dry}} \quad (2.5)$$

Here,  $W_{\text{swollen}}$  is the weight of the swollen gel, and  $W_{\text{dry}}$  is the weight of the dry gel.

The swelling kinetics of hydrogels can also be determined from the swelling kinetic curves. First, the weight of the dry gel ( $W_0$ ) is determined. The dried gel was then immersed in an excess amount of water until the swelling equilibrium was attained. The weight of the wet gel ( $W_t$ ) was determined after the removal of the surface water. The swelling ratio was calculated with the following equation.

$$\text{Swelling ratio} = (W_t - W_0)/W_0 \quad (2.6)$$

Many groups have investigated the swelling/shrinking kinetics of PNIPAAm gels when the temperature is increased or decreased to above or below the LCST, respectively. For example, Yoshida and coworkers have compared the shrinking kinetics of PNIPAAm gels with different architectures. Comb-type PNIPAAm hydrogels collapsed from a fully swollen state in less than 20 min, whereas similar gels without grafted side chains took more than one month to undergo full shrinking [41, 168]. They also reported a comb-type grafted hydrogel composed of PEO graft chains in the crosslinked PNIPAAm network [125]. The swelling characteristics are crucial to the use of hydrogels in biomedical and pharmaceutical applications since the equilibrium swelling ratio affects the solute diffusion coefficient, surface wettability and mobility and optical and mechanical properties of the hydrogel. The swelling properties are determined by many factors, including the type and composition of monomers, crosslinking density and other environmental factors such as temperature, pH and ionic strength.

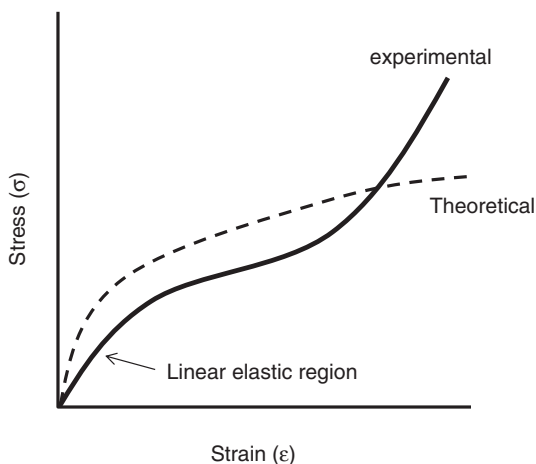
#### 2.4.4 Mechanical Properties

The mechanical properties of hydrogels depend on their composition and structure [169]. Generally speaking, the polymer gels are very weak, i.e., gels are soft and brittle, and the gel cannot with stand large deformation. This is mainly due to the fact that gels are far from fully connected polymer networks and contain various types of inhomogeneities, such as dangling chains and loops. Biopolymers, such as gelatin gels and polysaccharides, have been extensively investigated because of their variety of applications in products such as cosmetics and foods. The mechanical performance of conventional hydrogels can be expressed as elastic modulus. The elastic modulus can range from kPa to MPa, e.g., gelatin gel and agarose. The mechanical properties of these gels, however, have been mostly evaluated by shearing or compression, not by stretching, because of poor deformability.

Chemical gels, made via the copolymerization of a monomer in the presence of a crosslinker or by crosslinking of polymer chains, are also mechanically weak. The mechanical properties of the hydrogel are affected by the comonomer composition, crosslinking density, polymerization conditions and degree of swelling. The mechanical strength of the hydrogel is often derived entirely from the crosslinks in the system, particularly in the swollen state where physical entanglements are almost nonexistent. The dependence of mechanical properties on crosslink density has been studied intensively by many researchers. However, it should be noted that when the crosslinking density is altered, changes in properties other than strength also occur. Recently, new types of gels capable of large deformation have been developed. Okumura and Ito [170] developed a slide-ring (SR) gel (also called topological gel) by crosslinking polyrotaxane, which consists of PEG threaded through a ring molecule of cyclodextrin (CD). Because CDs are not covalently bonded to the axis polymer, the crosslinks can slide along the axial chain, and thus, the SR gels show unique mechanical and swelling properties. Gong et al. [171] have succeeded in creating double-network (DN) gels, which exhibit toughness and very large energy dissipation. For example, a DN composed of two mechanically weak hydrophilic networks, poly(2-acrylamido-2-methylpropanesulfonic acid) and polyacrylamide, provides a hydrogel with outstanding mechanical properties. Hydrogels containing about 90 % water possessed an elastic modulus of 0.3 MPa and fracture stress of ~10 MPa, demonstrating both hardness and toughness. This was explained by the effective relaxation of locally applied stress and the dissipation of crack energy through a combination of the different structures and densities of the two networks.

Many experimental methods were previously employed to characterize the mechanical properties, mainly Young's modulus of hydrogels. Common methods include simple tensile testing to determine the rubber elastic behavior or dynamic mechanical analysis under tension or shear to determine the viscoelastic properties. For most uniaxial tensile tests, the hydrogel samples are cut and prepared into a dumb bell shape and placed between two clamps [172]. Tests are run at constant extension rates with varying loads until the sample reaches ultimate failure. The stress-strain ( $\sigma$ - $\epsilon$ ) behavior of the samples can be obtained from these tests and the slope of this data would provide Young's modulus of the hydrogels. In addition ( $\sigma$ ) versus ( $\epsilon - 1/\epsilon^2$ ) can also be plotted, and using the rubber elasticity equations, the shear modulus ( $G$ ) can be obtained from the slope of the plot. Figure 2.9 shows the typical experimental stress-strain behavior of a crosslinked gel along with the results of theoretical statistical thermodynamic predictions. Compression testing is similar to tensile testing, except that instead of pulling the sample, it is compressed. The hydrogels are usually prepared as round samples, and compression tests are performed to plot the stress-strain curves. Young's modulus of the hydrogels is the slope of these curves. In theory, the values of Young's modulus obtained from tensile and compression tests for a particular hydrogel must be the same; however, it has been found that the values can differ. This could be attributed to the difference in the thickness of samples, which could lead to a difference in the diffusion of reactive species during polymerization.

**Fig. 2.9** Theoretical and experimental stress-strain curves for hydrogels



Atomic force microscopy (AFM) can be used not only for imaging the topography of surfaces, but also for measuring forces on a molecular level. To investigate the mechanical properties of soft matrices or thin films, the sample is compressed by the indenting AFM tip. The loading force is calculated from the deflection and spring constant of the cantilever. To calculate Young's modulus of the material, force-indentation curves are recorded and fitted to the Hertz model, which describes the elastic deformation of two spherical surfaces under load [173].

### 2.4.5 Rheology

The rheological properties are very much dependent on the types of structure (i.e., association, entanglement, and crosslinks) present in the system. Polymer solutions are essentially viscous at low frequencies and tend to fit the scaling laws:  $G' \sim \omega^2$  and  $G'' \sim \omega$ . At high frequencies, elasticity dominates ( $G' > G''$ ). This corresponds to Maxwell-type behavior with a single relaxation time, which may be determined from the crossover point, and this relaxation time increases with concentration. Crosslinked microgel dispersions exhibit  $G'$  and  $G''$  that are almost independent of oscillation frequency.

### 2.4.6 Surface Properties

The surface of a hydrogel can be rough, smooth or stepped; it can be composed of different chemistries or could be highly crystalline, disordered and inhomogeneous. Studies have been performed on the importance of roughness, wettability,

surface mobility, chemical composition, crystallinity and heterogeneity; however, significant research has not yet been performed on determining which parameters are of utmost importance in understanding biological responses to surfaces. Some of the techniques used for determining the surface property include electron spectroscopy, secondary ion mass spectrometry, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), scanning tunneling microscopy (STM) and atomic force microscopy (AFM). FTIR is a useful technique for identifying the chemical structure of a substance. This technique is widely used to investigate the structural arrangement in a hydrogel by comparison with the starting materials. SEM can be used to provide information about the sample surface topography, composition, and other properties such as electrical conductivity. This is a powerful technique widely used to visualize the characteristic ‘network’ structure in hydrogels. The information obtained through these methods can be used to monitor contamination, ensure surface reproducibility and explore the interaction of the hydrogels with living systems.

### ***2.4.7 Other Techniques***

The main methods used to characterize and quantify the amount of free and bound water in hydrogels are differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR). Proton NMR gives information about the interchange of water molecules between the so-called free and bound states. The use of DSC is based on the assumption that only the free water may be frozen, so it is assumed that the endotherm measured when warming the frozen gel represents the melting of the free water, and that value will yield the amount of free water in the hydrogel sample being tested. The amount of bound water is then obtained from the difference between the measured total water content of the hydrogel test specimen and the calculated free water content. Thermogravimetric (TG) analysis and X-ray diffraction are also used to confirm the formation of crosslinked network gel structures of hydrogels. Neutron scattering based techniques have been used to study the relationship of the structure of polymer gels and mechanical properties [174, 175].

## **2.5 Applications of Smart Hydrogels**

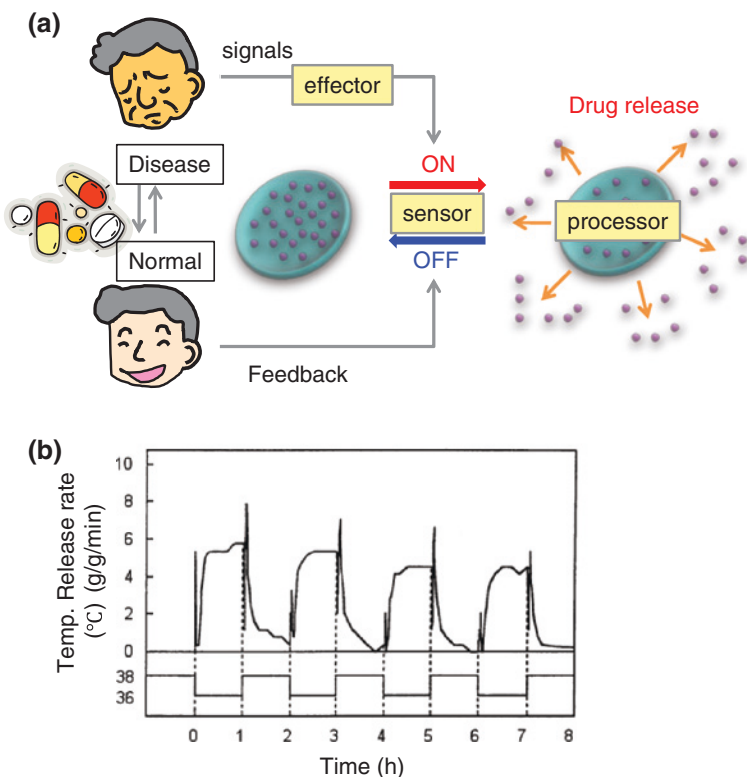
Hydrogels have received considerable attention in the last few decades owing to their exceptional promise in biomaterial applications. PHEMA was the first synthetic hydrogel to be synthesized in 1936 by DuPont scientists [176], and was established as an excellent candidate for contact lens applications by Wichterle and Lim [10]. Since then, hydrogels have been of great interest to biomaterial scientists. Some of the most successfully demonstrated applications are described in the following subsections.

### 2.5.1 'On-Off' Drug Delivery Systems

Well-designed drug delivery systems must control solute release over time. Various biomaterials have been investigated to control drug release; however, among them, hydrogels show two distinct advantages. (1) The rate of drug release can be controlled in many ways such as by changing the crosslinking density, preparing the hydrogel with monomers of controlled hydrophilicity, or controlling the ratio of hydrophilic to hydrophobic monomers. (2) Hydrogels may interact less strongly with drugs; consequently, a larger fraction of active molecules of a drug, especially proteins and peptides, can be released through hydrogel carriers. Better control over the delivery of drugs to specific sites in the body at specific times would reduce unwanted side effects and improve medical treatment dramatically (Fig. 2.10a). 'Smart' hydrogels are promising materials for controlling drug delivery, since they change their properties in response to specific stimuli. Temperature-responsive hydrogels have been studied most extensively to obtain an 'on-off' drug release profile in response to a stepwise temperature change. Okano et al. [177–179] have achieved complete and rapid 'on-off' regulation of drug permeation in response to stepwise temperature changes by using temperature-responsive hydrogels comprising PNIPAAm. A dense gel surface layer (skin layer) is established immediately after temperature increase above the hydrogel's collapse temperature. A possible explanation for this skin layer formation is that, at temperatures above its collapse point, the outermost gel layer interacts with its environment and then dehydrates quickly, forming a dense surface layer within seconds. The formed skin layer is dense enough to stop or retard the flux of water inside the gel to the outside of the gel. Gel surface skin layer formation can be controlled by changing the gel polymer chemistry, namely, the lengths of alkyl side chains on comonomers used during gel co-polymerization. Marked differences are observed in the initial shrinking process between three types of PNIPAAm-derived gels copolymerized with alkyl methacrylate comonomers, butyl methacrylate (BMA), hexyl methacrylate (HMA) and lauryl methacrylate (LMA) after temperature increase from 20 to 30 °C. P(IPAAm-*co*-HMA) and P(IPAAm-*co*-LMA) gels, both with longer alkyl side chains, shrank to only 20–30 % of their original volume observed at 20 °C, whereas P(IPAAm-*co*-BMA) gels shrank up to 80–90 % after equilibration. This result indicates that the rapid formation of a thin and dense skin layer can be regulated by selecting appropriate alkyl side chain lengths. With the longer alkyl side chains, denser skin layers were formed, preventing water efflux. The 'on-off' drug release in response to smaller temperature changes between 36 and 38 °C was also achieved, as demonstrated in Fig. 2.10b [180]. Both hydrophilic *N,N*-dimethylacrylamide (DMAAm) and hydrophobic BMA were incorporated into NIPAAm copolymer hydrogels, producing an LCST near 37 °C while maintaining high thermosensitivity. The rapid increase in release rate is attributed to a squeezing effect of drug molecules resulting from the shrinking of the gel surface region.

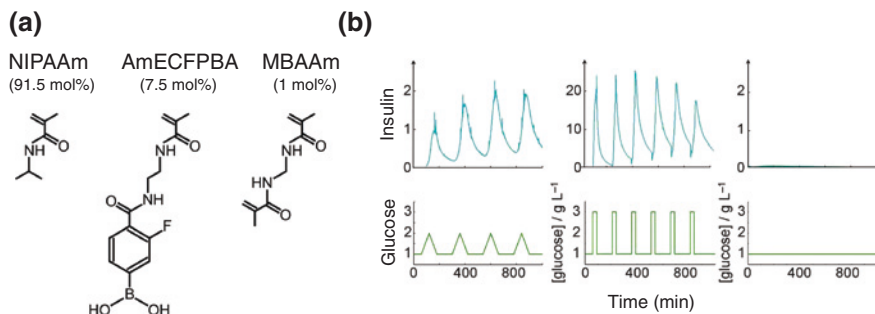
Another example of 'on-off' drug release control is achieved by using sugar-responsive gels for the possible treatment of diabetes mellitus. Pancreatic islets





**Fig. 2.10** **a** Design concept for 'smart' drug delivery systems with sensor, processor, and effector functions, which can respond directly to a person's individual needs. **b** 'On-off' drug release from temperature-responsive hydrogels in response to stepwise temperature changes between 36 and 38 °C [180]

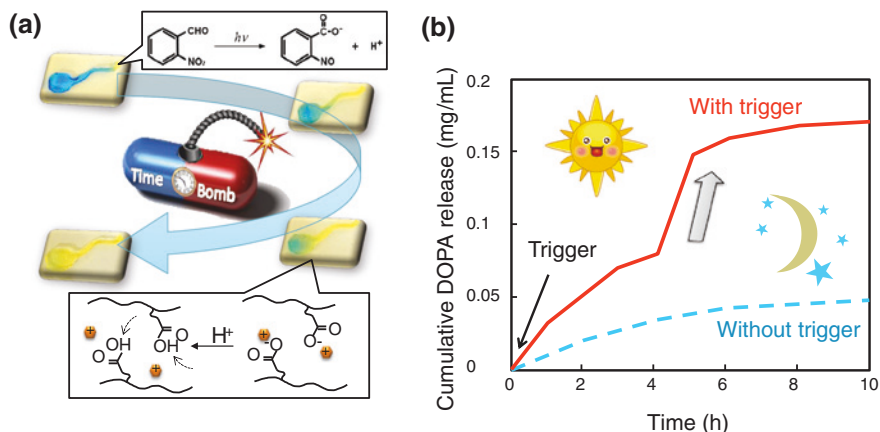
release insulin to lower the blood glucose level and regulate the glucose level within the range from 70 to 110 mg/dl by an autofeedback mechanism under healthy physiological conditions. It is necessary to externally administer exogenous insulin to diabetic patients (type I IDDM) who cannot control their blood glucose level. However, overdose may result in hypoglycemia and coma, which is a life-threatening state for these patients. Therefore, insulin must be carefully administered to avoid hypoglycemia in diabetic patients. Thus, in order to maintain a physiological glucose level, artificial systems sensing glucose and releasing appropriate amounts of insulin have been investigated. Kataoka et al. [154] have designed glucose-responsive gels composed of PNIPAAm derivatized with phenylboronic acid (PBA) groups as the glucose-sensing moiety. Significantly, PBA groups in aqueous solution are equilibrated between undissociated and dissociated forms, as shown in Fig. 2.8. Such equilibrium is shifted in the direction of increasing charged phenylboronates through complexation with glucose because only



**Fig. 2.11** **a** Chemical structures of monomers contained in glucose-sensitive hydrogels and their optimized molar amounts in the feed to obtain glucose sensitivity under physiological pH and temperature [155, 198]. **b** (Top) Time course of changes in fluorescence intensity of FITC-labeled insulin released from glucose-sensitive gels under physiological conditions. (Bottom) Temporal patterns of fluctuation in glucose concentration [16]

charged boronates form stable complexes with cis-diols such as glucose under aqueous conditions. The PNIPAAm-PBA gels have volume transition temperatures that shift with glucose concentration. Interestingly, the gels are in collapsed states below 100 mg/dL glucose and in swollen states above 300 mg/dL glucose at 28 °C and pH 9. Such a glucose concentration range corresponds to the normal glucose levels in our body. Glucose-responsive insulin release was also performed using NB gels. Matsumoto and coworkers have further developed this glucose-responsive hydrogel system to operate under physiological conditions (pH 7.4, 37 °C), aiming for future use in a self-regulated insulin delivery system to treat diabetes mellitus. The approach involves the use of a newly synthesized phenylborate derivative, 4-(1,6-dioxo-2, 5-diaza-7-oxamyl) phenylboronic acid (DDOPBA), possessing an appreciably low  $pK_a$  (~7.8) as a glucose-sensing moiety, as well as the adoption of PNIPMAAm as the main chain that exhibits critical solution behavior in the range close to physiological temperature (Fig. 2.11a) [155, 156]. Glucose- and pH-dependent changes in the critical solution behavior of the resultant copolymers were investigated at varying temperatures, revealing definite glucose sensitivities near the physiological conditions. The release of insulin from the gel has been continuously controlled by the skin layer with close correspondence to each addition pattern of glucose (Fig. 2.11b) [16].

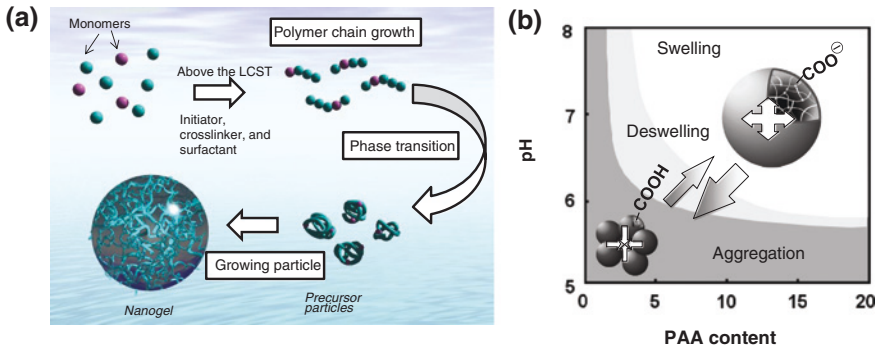
Brazel and Peppas [181] reported pulsatile drug-releasing hydrogels to create both pH- and temperature-triggered devices for coronary-thrombosis-induced heart attack and stroke patients. Thrombolytic and antithrombotic agents represented by heparin and streptokinase have minute-order half-lives in circulation and are only required when blood clots form and specifically only at the site of the clot. To produce a therapeutic device with responsive hydrogels, they synthesized P(IPAAm-co-MAA) gels and investigated the release profiles of biologically active agents as a function of pulsatile pH and temperature. Streptokinase release was seen only when the gels were exposed to low temperature below the



**Fig. 2.12** **a** Schematic of timed explosive drug release from pH-responsive hydrogels utilizing a phototriggered spatial pH-jump reaction [137]. **b** Drug release profiles of DOPA from gels with and without UV irradiation [130]

LCST and pH above  $pK_a$ . Drug release was observed to immediately decrease and completely stop after simultaneously decreasing the pH and increasing the temperature. After changes in both pH and temperature, the gel networks began to collapse. Their mesh sizes, representative of the space available for drug diffusion, dropped rapidly from approximately 12.5 to less than 10 nm, making it difficult for streptokinase of approximately 5.5 nm molecular diameter to diffuse through the collapsing pores. Heparin release from the gels, however, was not controlled because the mesh sizes of the gels were too large to control heparin diffusion (approximately 3 nm molecular diameter), even in the collapsed gel state.

Recently, the authors have developed an approach that could allow a more subtle control and timing of drug delivery. Although smart hydrogels are promising materials for controlling drug delivery, they usually require continuous stimulation to maintain these changes. We demonstrated the control of the acidity inside a pH-responsive hydrogel by loading it with a compound called NBA (Fig. 2.12a) [137]. This releases protons, which increase the acidity, when irradiated with UV light. When an NBA-loaded hydrogel was irradiated, the acidity increased inside; if only part of the gel was irradiated, the acidity throughout increased gradually as protons diffused. We loaded a hydrogel with NBA and L-DOPA, a precursor of the brain chemical dopamine that is used in the treatment of Parkinson's disease. The change in acidity of the gel upon UV irradiation caused L-DOPA to be released because the acidity disrupted the interaction of L-DOPA with the molecules in the gel [130]. Irradiation with UV not only enhanced the overall L-DOPA release from the hydrogel, but also caused an extra 'explosive' release five hours after irradiation (Fig. 2.12b). This allowed the drug release to be timed, as well as triggered, in a controlled manner. Being able to control the release of drugs from hydrogels by triggering a change in acidity could help indesigning programmable drug delivery techniques that offer improved targeting of treatment.



**Fig. 2.13** **a** Schematic illustration of precipitation polymerization method used for preparation of smart nanogels. **b** Swelling-deswelling-aggregation diagrams for P(NIPAAm-*co*-PAAC) nanogels [184]

Smart hydrogels in the form of microgels and nanogels have also been developed by many researchers, because they display many advantages when they are used in biological applications. PNIPAAm gel particles are typically prepared by precipitation polymerization or emulsion polymerization [182, 183]. In the precipitation polymerization approach, the polymerization of monomers and crosslinkers is initiated in water by a free-radical initiator at temperatures above the LCST. Once the growing polymer chains reach a critical length, they collapse and phase separate to form colloidal particles (Fig. 2.13a). This method offers numerous advantages, such as the production of highly uniform particles and the ability to control particle parameters, such as the size, charge, and crosslinking density. Omura et al. [184] developed a smart nanogel system that fully swells at normal physiological pH values but undergoes a sharp volume phase transition at moderately acidic pH values. The nanogels were prepared by precipitation polymerization of NIPAAm and PAAc in the presence of the crosslinker, MBAAm. At room temperature, the NIPAAm-PAAc nanogels were discrete, spherical structures with diameters ranging from 200 to 250 nm. The hydrodynamic diameter of the nanogels decreased to ca. 100–150 nm when the solution temperature was increased to 37 °C. At 37 °C, when the  $pK_a$  was below that of the NIPAAm-PAAc, the gels collapsed and aggregated. However, at 37 °C and a physiological pH of 7.4, the nanogels did not fully collapse owing to the charge–charge repulsion derived from the ionized carboxyl groups of the PAAc (Fig. 2.13b). Thus, such nanogel particles could be useful for releasing drugs in regions of local acidosis, including sites of infection, tumors, ischemia, and intracellular endosomes.

### 2.5.2 Injectable Hydrogels

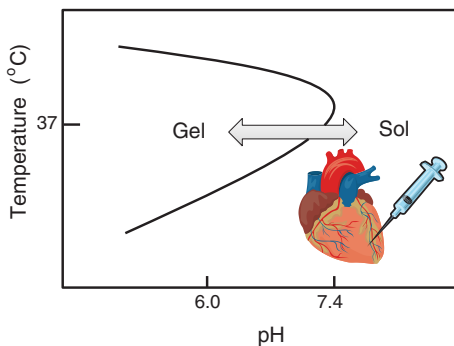
One of the most obvious ways to provide sustained-release medication is to place the drug in a delivery system and inject or implant the system into the body tissue. Injection of an in situ gel-forming biopolymer is thus becoming increasingly

attractive for the development of therapeutic implants and vehicles [185, 186]. Copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) (known as poloxamers) in aqueous solutions are well-known thermoset gel-forming materials *in situ* [187], but lack physiological degradability and induce unexpected increases in the level of plasma cholesterol or triglycerides in rats when injected intraperitoneally. To add a biodegradable capacity, the PPO segment of PEO–PPO–PEO block copolymers is often replaced by a biodegradable PLLA [51] or PLGA [52] segment. When low-molecular-weight PEG versus high-molecular-weight PLGA is used, the aqueous solution of PEG–PLGA–PEG triblock copolymer forms a solution at room temperature, whereas, at body temperature, it becomes a gel within a few seconds. The molecular architecture was not limited to the A-B-A-type block copolymer, but expanded into 3D, hyper branched structures, such as a star-shaped structure [53]. Chenite et al. [56] developed novel thermally sensitive combinations of chitosan/polyol salts, which turn into gel implants when injected *in vivo*. These formulations possess a physiological pH and can be kept as liquid below room temperature for encapsulating living cells and therapeutic proteins; they form monolithic gels at body temperature. When injected *in vivo*, the liquid formulations turn into gel implants *in situ*. This system was used successfully to deliver biologically active growth factors *in vivo* as well as an encapsulating matrix for living chondrocytes for tissue engineering applications.

Thermosensitive, specific-ion-sensitive or pH-sensitive hydrogels have been examined for their potential as vehicles for ocular drugs. The eye presents a challenge in the development of sustained or controlled release systems owing to its sensitivity and effective protection mechanisms, such as lacrimal secretion and blinking reflex, which cause rapid drainage of bioactive agents after topical administration. Therefore, *in situ* gels are preferred since they are conveniently dropped as a solution into the eye, where they undergo transition into a gel. Poloxamers, as thermogelling polymers, could be applicable for the development of effective ophthalmic drug delivery [188]. Ion-sensitive polymers belong to the group of *in situ* gelling materials mainly used for ocular drug delivery. The presence of alginate polymers significantly extended the duration of the pressure-reducing effect of pilocarpine to 10 h, allowing only once a day administration in the case of carteolol [189].

Recently, a number of researchers have investigated the use of pH as a stimulus for reversible gelation in polymeric systems [190–192]. The pH-sensitive system could facilitate drug delivery to regions of local acidosis, including sites of infection, neoplasia, or ischemia. The incorporation of carboxylic acid-derived monomers, such as AAc or MAAC, has been carried out to impart pH sensitivity in a variety of copolymers. With  $pK_a$  values lower than the physiologic pH of 7.4 ( $pK_a$  4–5), these polymers can be designed to target acidic regions. However, under physiological conditions, the very low  $pK_a$  values of PAAc and PMAAC generally limit the use of these polymers for drug targeting to very low pH systems such as the stomach. Hoffman and colleagues have reported that the longer alkyl segments on PAAc raise the carboxylate  $pK_a$  and facilitate sharp phase transitions at pH values greater than pH 6.0 [193, 194]. They have developed injectable NIPAAm-PAAc copolymers for the delivery of angiogenic growth factors [149]. NIPAAm-PAAc copolymers undergo sharp, reversible gelation at intermediate

**Fig. 2.14** Typical phase diagram of pH- and temperature-responsive block copolymers in solution. These types of copolymer could be useful in providing sustained delivery of therapeutic molecules to regions of local acidosis such as ischemic tissue



acidic pH ( $\sim$ pH 5–6) but remain soluble at normal physiologic pH (7.4). This pH response could first promote gel formation in diseased tissue exhibiting local acidosis, and second, promote polymer dissolution and elimination once the tissue has returned to normal physiologic pH. Such a system could be useful in providing sustained delivery of therapeutic molecules to regions of ischemia, such as in therapeutic angiogenesis that has the potential to promote healing of ischemic tissue (Fig. 2.14).

The vagina, in addition to being an important organ of the reproductive tract, serves as a potential route for drug administration. It has been reported that 30–50 % of vaginitis episodes are due to *Candida* infection and that two-thirds of all women experience acute episodes of vaginal candidiasis at least once during their lifetime [195]. For vaginal delivery systems of antifungal agents to be more effective, they need to reside at the sites of infection for a prolonged period. Formulations based on a thermoplastic graft copolymer that undergoes in situ gelation have been developed to enable the prolonged release of active ingredients such as nonoxynol-9, progestins, estrogens, peptides and proteins [196]. This polymer offered prolonged antifungal activity over several days against *Candida albicans* vaginitis while reducing the toxicity of the drug on epithelial cells.

### 2.5.3 Tissue Engineering

Tissue engineering has emerged as a promising technology for the design of an ideal, responsive, living substitute with properties similar to those of the native tissue [19]. Scaffolds play an important role in scaffold-guided in vitro tissue engineering. Scaffolds are basically 3D structural templates that support cell adhesion, migration, differentiation, and proliferation and provide guidance for neotissue formation. Hydrogels in particular have emerged as useful scaffolding biomaterials as they most closely resemble the natural tissues. Moreover, the aqueous environment provided by hydrogels mimics those of cells in the body. Both synthetic and natural hydrogels are used as scaffolds for tissue engineering in order

to repair cartilage, tendon, ligament, skin, blood vessels and heart valves [6]. The synthetic hydrogels focused on for use as scaffolds are polyurethanes (PU), PEO, PNIPAAm, PVA, PAAc and poly(propylene furmarate-*co*-ethylene glycol) [P(PF-*co*-EG)], whereas naturally derived hydrogels are agarose, alginate, chitosan, collagen, fibrin, gelatin, and hyaluronic acid (HA). Injectable hydrogels are also promising substrates for tissue engineering applications owing to their high tissue like water content, ability to homogeneously encapsulate cells, efficient mass transfer, easily manipulated physical properties and minimally invasive delivery [6, 197]. The hydrogel precursor loaded with growth factors or targeted cells can be injected into the wound site, where it undergoes a sol-gel transition in situ owing to physical or chemical stimuli.

Matsuda and coworkers have designed a thermoresponsive cell-adhesive matrix using PNIPAAm-grafted gelatin [198] and HAs [199]. The PNIPAAm-grafted HAs were water soluble at room temperature, where as they precipitated at temperatures above approximately 34 °C in water. Copolymers composed of HA and PNIPAAm have also been prepared by Ha et al. [200] to create temperature-sensitive injectable gels. Semi-telechelic PNIPAAm, with amino groups at the end of each main chain, was synthesized by radical polymerization using 2-aminoethanethiol hydrochloride (AESH) as the chain transfer agent, and was then grafted onto the carboxyl groups of HA using carbodiimide chemistry. The result of the thermo-optical analysis revealed that the phase transition of the PNIPAAm-grafted HA solution occurred at approximately 30–33 °C. PNIPAAm-grafted HA exhibited an increase in viscosity above 35 °C, thus allowing the gels to maintain their shape for 24 h after in vivo administration. Stile et al. [201] have developed NIPAAm-based hydrogels that support tissue formation in vitro. Loosely crosslinked PNIPAAm and P(NIPAAm-*co*-AAc) hydrogels were synthesized with *N,N'*-methylenebis(acrylamide) crosslinker. At room temperature, the hydrogels were transparent and extremely pliable, where as at 37 °C, the matrices became opaque and were significantly more rigid. The P(NIPAAm-*co*-AAc) hydrogel demonstrated significantly less volume change between room temperature and 37 °C, contained significantly more water at 37 °C, and had an LCST that was significantly higher than that of the PNIPAAm hydrogel. The hydrogels supported bovine articular chondrocyte viability for at least 28 days of in vitro culture, and cartilage-like tissue was formed in the matrices. These hydrogels can be injected through a small-diameter aperture and offer the benefit of in situ stabilization without the possible deleterious effects of in situ polymerization.

Smart hydrogels can also be applied to cell encapsulation. Cell technology provides a promising therapeutic modality for diabetes, hemophilia, cancer and renal failure [202–204]. The selection of a suitable biomaterial as a membrane for encapsulating cells is the major challenge to be overcome to enable successful cell encapsulation therapy. Biocompatibility, microporous structure, and minimal surface irritation within the surrounding tissues of hydrogel have made them attractive for this application. They can be designed with the required porosity that resists any entrance of immune cells while allowing stimuli, oxygen, nutrients, and waste transfer through the pores. Genetically modified alginates and polyethylene



oxide-based hydrogels have been studied as cell encapsulation systems. Most problems of the in situ gelation system, however, lie with the reagents and the by-products of crosslinked hydrogels, which have the potential to be toxic to cells [205]. Increased cell death has been observed with a high concentration of exposed unreacted side chains after gelation [206]. Moreover, release of the encapsulated cells from vehicles is a difficult, potentially harmful process. The authors have recently developed temperature-responsive crosslinked nanofibers and demonstrated the ability to capture, encapsulate, and release cells by dynamically transforming the fibrous structure of the nanofibers into hydrogel-like structures by wrapping, swelling, and shrinking processes in response to alterations of external temperature [31]. By using external signals, cell capture, encapsulation, and release were successfully demonstrated. The released cells show excellent viability and proliferation behavior. This study extends the capture and release methods to a variety of bioactive compounds, the activity of which can be controlled by switching their accessibility to the environment. Further functionalization of the nanofibers could also be used for the immobilization of peptides or antibodies, which are highly promising for the separation, purification, preservation, and delivery of the target molecules and cells.

ECM-mimicking enzyme-responsive hydrogel scaffolds that permit cell migration have also been developed by Lutolfand et al. [207, 208]. They used oligopeptides as crosslinkers in PEG-based hydrogels. The peptide sequences are cleavable by matrix metalloproteinases (MMPs) to form a gel into which cells can infiltrate. MMPs are a family of enzymes that play many roles including the breakdown of ECM molecules during tissue remodeling and disease. Therefore, the integration of MMP-cleavable sites is a logical approach toward ECM mimics. The potential for bone tissue engineering was tested by loading the gel with bone morphogenetic protein-2 (BMP-2), which is known to be involved in bone formation. An assessment of the degradation behavior of MMPs and the cell invasion of provisional matrices revealed that the healing response in vivo depends on the enzymatic sensitivity of the matrix. Kim et al. [209] have also created an injectable hydrogel of NIPAAm-AActo mimic the ECM. These hydrogels are prepared by crosslinking an MMP-13/collagenase-3-degradable peptide sequence and NIPAM in the presence of Arg-Gly-Asp-modified PAAc. The proteolytic degradation and cell adhesion properties of this hydrogel were studied using rat calvarial osteoblasts. Collagenase was found to degrade the hydrogel at a rate dependent on the concentration of collagenase in relation to the PAAc chain. There is an increase in cell migration in MMP-degradable hydrogels compared with nondegradable gels, indicating the advantage of bioresponsive hydrogels.

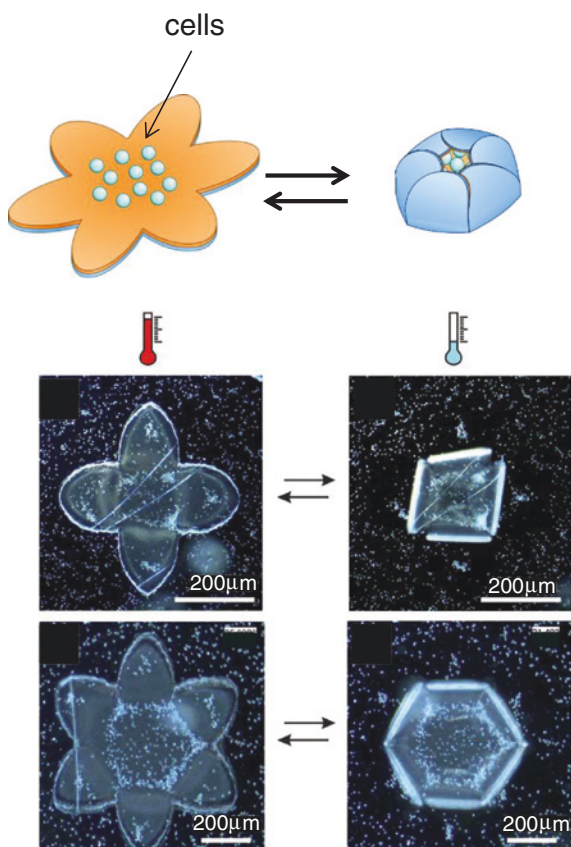
### **2.5.4 Actuators**

Smart hydrogels have also been applied to biomimetic actuators or artificial muscles because polymers that undergo dimensional changes in response to various

environmental stimuli are capable of transducing chemical or physical energy directly into mechanical work. Conducting polymers, such as polypyrrole (PPy), polythiophene, and polyaniline, have attracted considerable attention because dimensional changes resulting from electrochemical doping and dedoping in an electrolyte solution or in a swollen state can be applied to produce electroactive polymer (EAP) actuators or artificial muscles [210, 211]. Osada et al. [24] reported electrically driven systems comprising polymer gels and electrodes that were actuated to bend and stretch repeatedly in response to alternating voltages, whereby the mechanical motion of these gels was in fact driven by the direction of the electric field. Okuzaki et al. [212, 213] have reported that electrochemically synthesized PPy films contracted in air under the application of an electric field. The motion of the film is driven by the reversible absorption of water vapor.

Temperature-responsive PNIPAAm-based actuators that bend in response to a temperature change have been reported as well. Hu et al. [214] described partially interpenetrated polymer networks composed of PNIPAAm and poly(acrylamide) (PAAm) hydrogels, so-called “bi-gels”, which bend into circles in response to increasing temperature. These “bi-gels” grasp or release an object simply by adjusting the water temperature. Stoychev et al. [215] have reported the fabrication of 3D microobjects by the controlled folding/bending of a thin film, i.e., “microorigami”. In this approach, two polymers were used. The first one is thermoresponsive PNIPAAm. In aqueous media, PNIPAAm reversibly changes its solubility at 33 °C. The second polymer is hydrophobic and water-insoluble poly( $\epsilon$ -caprolactone) (PCL). Both polymers were deposited on a substrate in the form of a crosslinked bilayer. Swelling and shrinking of PNIPAAm resulted in reversible rolling of the bilayer and enabled the formation of tubes. Four- and six-arm self-folding capsules were fabricated and applied for reversible encapsulation of yeast cells (Fig. 2.15). Jeong et al. [216] have also constructed reversible color- and shape-tunable photonic actuators by transforming the programmed 2D structures to the 3D objects via the bending, twisting and folding mechanisms. A thermally curable hydrophobic poly(dimethylsiloxane) (PDMS) and a UV-curable hydrophilic PU/HEMA elastomeric blending precursor are selected as constituents because of their flexibility, optical transparency and dramatically different swelling responses to selected solvents. By changing the geometrical factor, selected materials and polarity of the solvents, the desired shape and color of the scrolled, helical, and cubic actuators can be achieved. When the folding, bending, and twisting technologies are combined with lithographic patterning technology in micrometer length scale, this unique technique may have great potential for applications in mechanical actuators and optoelectronic and biomimetic devices. He et al. demonstrated this concept on the example of a millimeter-size PMMA-PHEMA bilayer with an attached mucoadhesive drug layer. The nonswelling PHEMA layer serves as a diffusion barrier, minimizing any drug leakage in the intestine [217]. The resulting unidirectional release provides improved drug transport through the mucosal epithelium. The functionality of this device is successfully demonstrated *in vitro* using a porcine small intestine. Asoh et al. [22] reported a novel strategy for the preparation of thermoresponsive bending gradient

**Fig. 2.15** Encapsulation of yeast cells inside the self-folding star-shaped polymer bilayer. Yeast cells are adsorbed on the polymer bilayer at elevated temperature. Cooling leads to swelling of the thermoresponsive polymer and folding of the capsules. Further heating results in unfolding of the capsules and release of the cells [215]

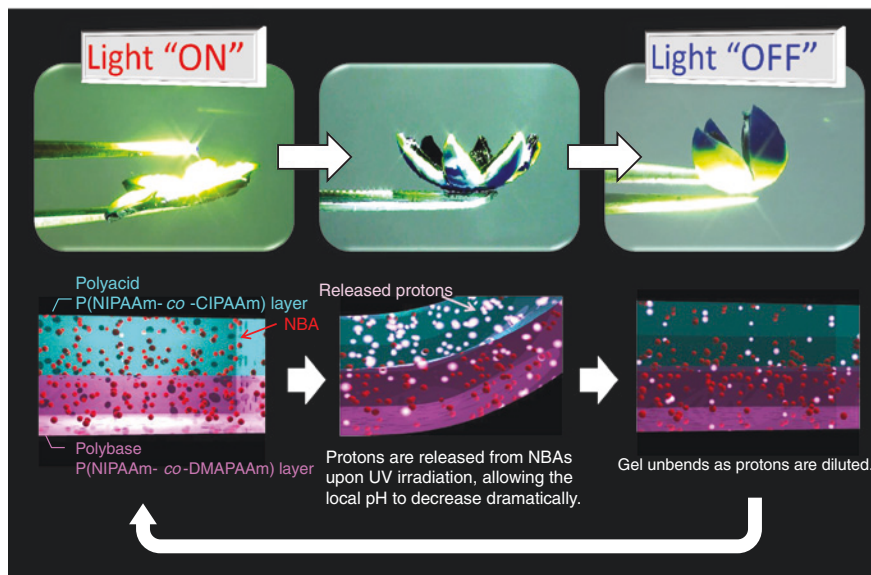


gels. PNIPAAm gels with two types of nanostructured gradients, consisting of either silica nanoparticles or nanopores, showed uniquely different bending properties depending on their shrinkage characteristics. These gradient gels were simply fabricated through electrophoresis and subsequent photo-polymerization. Differences in the physical properties between the two sides of the gradient gels are the driving force behind the bending of the gels. Zhang et al. have reported near-IR optically responsive hydrogels using single-walled carbon nanotube (SWNT)-PNIPAAm composite hydrogels. They demonstrated a well-defined, ultra fast response of the SWNT-PNIPAAm hydrogel actuators to near-IR laser excitation, making this design viable for many optically triggered applications [218].

In addition to physical signal-responsive systems, there are also several examples of bilayer systems that fold in response to chemical stimuli. The use of polymers sensitive to chemical signals allows the design of biomimetic actuators folding in response to specific chemical signals. Among them, pH-responsive systems are particularly important because the human body presents variations in pH along the gastrointestinal tract, in specific tissues (and tumoral areas) and intracellular compartments. pH-sensitive self-folding materials are commonly designed

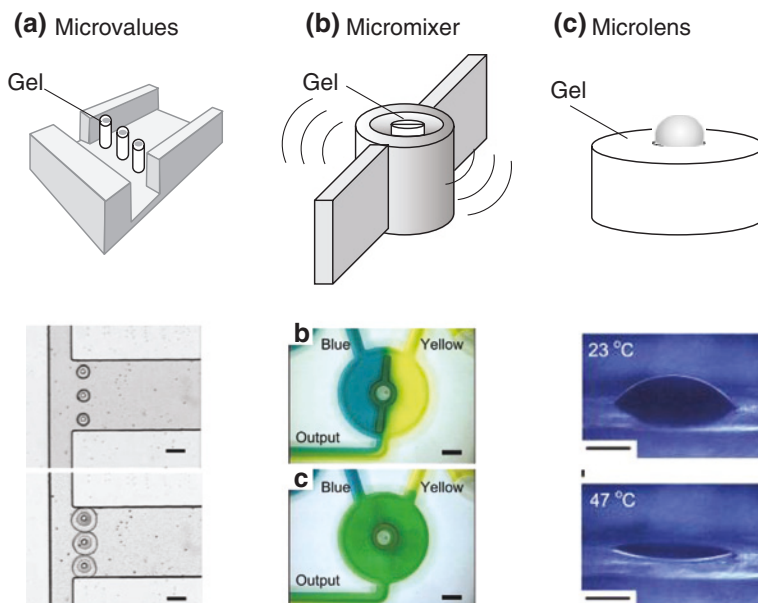
using weak polyelectrolytes as active polymers. Their ionic groups are counter balanced with oppositely charged ions that gradually diffuse into/out of polymer networks to induce spontaneous changes in their characteristics with temporal activation, which is observed in living systems, such as muscle and ciliary movement, pulsatile secretion of hormone, and brain waves. Luchnikov and coworkers demonstrated that the polystyrene (PS)-poly(4-vinyl pyridine) (PVP) bilayer [219], as well as the PS-PVP-PDMS trilayer [220], can roll at low pH when PVP is protonated and swells in water. The use of layers with a two-dimensional gradient of thickness allowed a thorough investigation of the folding. Bassik et al. [221] fabricated millimeter-size PEG/P(NIPAAm-*co*-AAc) bilayers that can snap in response to the pH signal. In contrast to physical stimuli that can easily penetrate through materials (e.g., heat, light, magnetic field, etc.), however, changing the pH quickly and precisely at a particular location in the system has been a major challenge, particularly inside hydrogels. From these perspectives, the authors focused on the photoinitiated proton-releasing reaction of ‘photoacid generators’ (PAGs), the  $pK_a$  of which, in an excited state, is significantly different from that in the ground state. We have successfully integrated the PAG into pH-responsive P(NIPAAm-*co*-CIPAAm) hydrogels to demonstrate rapid proton release upon UV irradiation, resulting in the decrease of intragel pH to below the  $pK_a$  of P(NIPAAm-*co*-CIPAAm) [130, 137]. We have demonstrated photo induced reversible control of self-bending using PAG-integrated pH-responsive bilayer hydrogels consisting of a polyacid P(NIPAAm-*co*-CIPAAm) layer and a poly base P(NIPAAm-*co*-*N,N'*-dimethylaminopropylacrylamide: DMAPAAm) layer [222]. The adhesion of these two layers was achieved by employing a semi-IPN using linear PAAc and branched poly(ethyleneimine) (PEI) chains, which form polyion complexes at the interface of the two gels via electrophoresis [25]. Reversible bending was successfully demonstrated in response to ‘on-off’ UV irradiation (Fig. 2.16). Additionally, self-bending of the non-UV-irradiated region of the gel was also achieved because the generated protons gradually diffused toward the nonirradiated region. The proposed system can be potentially applied in the fields of mechanical actuators, controlled encapsulation and drug release, robotics and microfluidic technologies because control over autonomous motion by both physical and chemical signals is essential as a programmable system for real biomedical and nanotechnological applications.

Smart hydrogels have also been incorporated into microfluidic devices to reduce the system complexity [223]. Microfluidics is the science and technology of designing and manufacturing devices that deal with the behavior, precise control and manipulation of small volumes of fluids [224]. Although recent progress in microfabrication techniques such as multilayer soft lithography enables us to design sophisticated microchips with hundreds of independent valves, most microfluidic materials themselves still lack stand-alone abilities. Therefore, it becomes increasingly apparent that on-demand switchable materials that can respond to external stimuli or their environment to produce dynamic and reversible change in critical properties have enabled progress in a growing number of diverse applications including bio/chemical analysis, chemical synthesis, cell



**Fig. 2.16** Photoinduced reversible self-bending/straightening of PAG-integrated pH-responsive bilayer hydrogels consisting of polyacid P(NIPAAm-co-CIPAAm) and polybase P(NIPAAm-co-DMAAAm) layers. The bilayer gels bend upward to the polyacid side when the light is turned on ('on' stage) because protons are released from PAG, allowing the local pH to decrease dramatically. After the UV light is turned off ('off' stage), the gels straighten to the original shape as protons are diluted [25, 222]

manipulation, biomedical monitoring, and point-of-care clinical diagnostics. From these perspectives, 'smart' microfluidic systems have been extensively studied using stimuli-responsive materials because they can receive device-generated signals and act as switches by themselves. Beebe et al. [26], for example, used a pH-responsive hydrogel-based valve that opened or closed depending on the pH of the flowing solution (Fig. 2.17a). Bistrip valves and arrowhead-shape valves have also been reported to allow flow in only one direction [225, 226]. Autonomous micromixers and micropumps have also been developed using pH- and temperature-responsive hydrogels, electroplated nickel (Ni) impellers, and magnetic stirrers (Fig. 2.17b) [227]. The Ni impeller was coupled with an underlying rotating magnetic stirrer that was constantly on. When the local environment pH was decreased, the hydrogel ring shrank, allowing the Ni impeller to rotate freely; however, when the local pH was raised above its transition point, the hydrogel ring expanded into a mushroom cap shape that exerts both downward and lateral forces on the Ni impeller, thus stopping the rotation of the Ni impeller. The 'smart liquid microlens' concept with the temperature-sensitive NIPAAm hydrogel that expands at low temperatures and contracts at high temperatures has also been demonstrated (Fig. 2.17c). In this system, the meniscus between water and oil was used as an optical lens and its focal length was adjusted by changing the curvature of

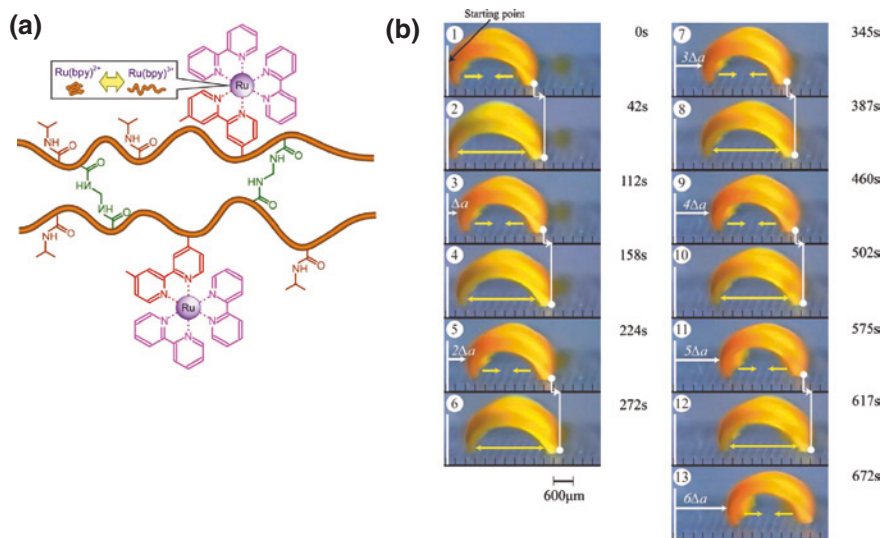


**Fig. 2.17** Autonomous microfluidics with smart hydrogels. **a** Autonomous flow control in microfluidic channels using pH-sensitive hydrogel valves [26]. **b** Autonomous microfluidic mixers using a pH-responsive hydrogel [227]. **c** Smart microlenses. The water–oil interface forms the liquid microlens. The microchannels allow the flow of fluids to the microlens structure [223]

this meniscus. The authors have recently described a facile approach to fabricate “smart” microfluidic channels that demonstrated a shape-memory-driven geometric switch and a new mechanism of flow control. We present on-demand switchable microchip materials that display potent rewritable and shape-memory properties, which are shown to contribute to fluidic control as pumps and valves [27]. This new class of smart microfluidic control techniques enables portable microfluidic-based diagnostic tools for biomedical applications and environmental monitoring with on-site analysis capability.

While stimuli-responsive polymers were designed to receive device-generated signals, which can act as switches, there has also been a dynamic growth in interest in self-actuating materials in recent years. Feinberg et al. [228] have demonstrated a self-walking bioactuator using biohybrid materials of engineered tissues and synthetic polymer thin films. They cultured cardiomyocytes on PDMS thin films micropatterned with ECM proteins to promote spatially ordered 2D myogenesis. The centimeter-scale constructs performed functions as diverse as gripping, pumping, walking, and swimming with fine spatial and temporal control and the generation of specific forces as high as 4 millinewtons per square millimeter. Yoshida and Okano have [229] successfully shown a novel biomimetic gel actuator that can walk spontaneously with a worm like motion without switching of external stimuli. The self-oscillating motion is produced by dissipating chemical energy





**Fig. 2.18** **a** Chemical structure of P(NIPAAm-co-Ru(bpy)<sub>3</sub>) gel. **b** Time course of self-walking motion of gel actuator (odd number: bending process at reduced state; even number: stretching process with propagation of chemical wave) [23]

from an oscillating reaction, that is, the BZ reaction [230, 231] occurring inside the gel. They prepared a copolymer gel of NIPAAm in which ruthenium(II) tris-(2, 2'-bipyridine) ( $\text{Ru}(\text{bpy})_3^{2+}$ ), a catalyst for the BZ reaction, is covalently bonded to the polymer chain (Fig. 2.18a). The P(NIPAAm-co-Ru(bpy)<sub>3</sub>) gel swells and deswells at the oxidized and reduced states of Ru(bpy)<sub>3</sub>, respectively. The BZ reaction in the gel generates periodic redox changes of Ru(bpy)<sub>3</sub>, and the chemical oscillation induces mechanical oscillation of the polymer network [232]. Although the gel is completely composed of synthetic polymer, it shows autonomous motion as if it is alive. To cause an anisotropic contraction with curvature changes, a gel strip with a gradient structure was prepared. By coupling with a ratchet mechanism, the gel walks with repeated bending and stretching motions by itself like a looper at a speed of 170  $\mu\text{m}/\text{min}$  (Fig. 2.18b) [23]. This “self-walking” gel actuator could serve as a new frame work for a biomimetic robot.

### 2.5.5 Sensors

Biomolecule-sensitive hydrogels that undergo swelling changes in response to specific biomolecules can be modified to design smart hydrogels that could degrade in response to an increase in the concentration of specific biomolecules. For example, the widely researched glucose-sensitive hydrogels have the ability to sense the levels of blood glucose and release insulin in accordance with the



glucose levels [155, 156, 233, 234]. NIPAAm copolymer microgels have been shown to be an excellent platform for designing label-free glucose-sensing materials. Sorrell and Serpe [235] reported that aminophenylboronic acid (APBA)-functionalized P(NIPAm-*co*-AAc) microgels in an etalon respond to 3 mg/mL glucose concentrations by red shifting their reflectance peaks by 110 up to 150 nm. Additionally, APBA-functionalized microgels have a depressed volume phase transition temperature of 18–20 °C, which shifts to 24–26 °C after glucose binding. These materials showed a marked visual color change, which is a first step towards developing direct-readout sensor devices. Wang et al. reported on the fabrication of multifunctional ratiometric probes for glucose and temperatures based on thermo responsive PNIPAAm microgels covalently incorporated with APBA, fluorescence resonance energy transfer (FRET) donor dyes, 4-(2-acryloyloxyethylamino)-7-nitro-2, 1, 3-benzoxadiazole (NBDAE), and rhodamine B-based FRET acceptors (RhBEA) [236]. The spatial proximity of FRET donors and acceptors within microgels can be tuned via thermo-induced microgel collapse or glucose-induced microgel swelling at appropriate pH and temperatures, leading to the facile modulation of FRET efficiencies. APBA moieties within P(NIPAM-APBA-NBDAE-RhBEA) microgels can bind with glucose at appropriate pH to form cyclic boronate moieties that can decrease the  $pK_a$  of APBA residues and increase the volume phase transition temperature of microgels.

Miyata and coworkers have reported a specific antigen-sensing semi-interpenetrating (semi-IPN) hydrogel network [32, 79, 237]. The hydrogel was fabricated by first polymerizing the vinyl-conjugated form of goat anti-rabbit (GAR) IgG (i.e., GAR IgG coupled to *N*-succinimidylacrylate) and then copolymerizing GAR IgG with vinyl-modified rabbit IgG, in the presence of the crosslinker, MBAAm. Noncovalent crosslinking between grafted antigens and antibodies resulted in shrinking of the hydrogel network in the absence of free antigens in the system. However, when free antigens were present in the system, the hydrogel network swelled owing to the rupture of the antigen-antibody crosslinks. This was due to competitive binding (to the immobilized antibodies) exhibited by the immobilized and free antigens in the solution. They also reported tumor-marker-responsive gels that exhibited volume changes in response to a tumor-specific marker glycoprotein ( $\alpha$ -fetoprotein, AFP) [157]. The glycoprotein-imprinted gel shrank in response to a target glycoprotein but a nonimprinted gel swelled slightly. The glycoprotein-responsive shrinking of the imprinted gel was caused by the formation of lectin-glycoprotein-antibody complexes that acted as reversible crosslinking points. As the shrinking behavior of biomolecularly imprinted gels in response to glycoproteins enables the accurate detection and recognition of tumor-specific marker glycoproteins, they have many potential applications as smart devices in sensing systems and for molecular diagnostics.

A hydrogel membrane sensitive to the metabolite nicotinamide adenine dinucleotide (NAD) and containing immobilized ligands and receptors was also investigated for the controlled diffusion of model proteins [238]. Both cibacron blue (ligand) and lysozyme (receptor) were covalently linked to dextran. NAD serves as a competing ligand and competes with cibacron blue in its interaction with

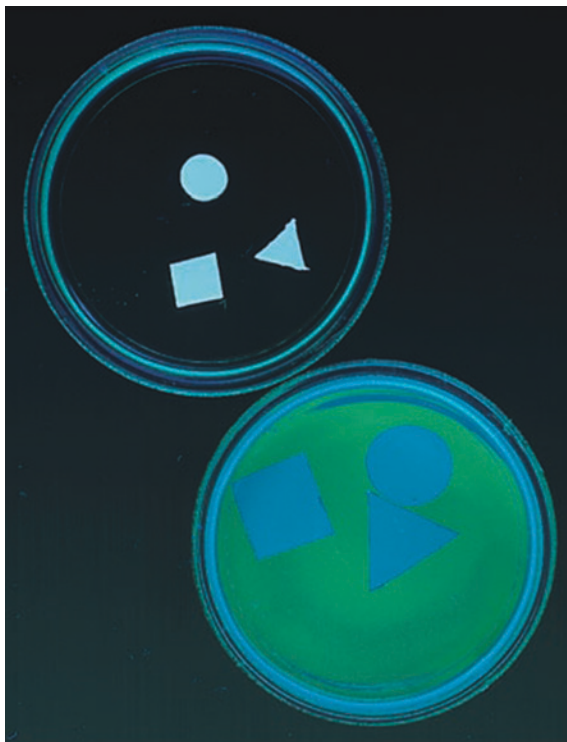
lysozyme. With the use of cytochrome C and hemoglobin as model proteins to examine the diffusion across the hydrogel membrane in response to differential concentrations of NAD, saturation kinetics was observed. This approach of sensing ambient levels of NAD can be generalized to diagnose the levels of different analytes by suitably selecting a competing ligand-receptor interaction, thereby affecting the permeability of the polymer membrane.

Molecular imprinting is a versatile method for creating macromolecular matrices (hosts) that display selective molecular recognition behavior. This is achieved by enabling the synthetic hosts to “memorize” the out fits of targeted guests. Oya et al. were pioneers in proposing the creation of stimuli-sensitive gels with the ability to recognize and capture target molecules using polymer networks consisting of at least two species of monomers, each playing a different role. One forms a complex with the template (i.e., the functional or absorbing monomers capable of interacting ionically with a target molecule), and the other allows the polymers to swell and shrink reversibly in response to environmental changes (i.e., a smart component such as NIPAAm). The gel is synthesized in the collapsed state and, after polymerization, is washed in a swelling medium. The imprinted cavities develop affinity for the template molecules when the functional monomers come into proximity, but when they are separated, the affinity diminishes. The proximity is controlled by the reversible phase transition that consequently controls the adsorption/release of the template (Fig. 2.19) [239]. The design of a precise macromolecular chemical architecture that can recognize target molecules from an ensemble of closely related molecules has a large number of potential applications. The main thrust of research in this field includes separation processes, immunoassays and biosensor recognition elements.

### 2.5.6 Self-Healing

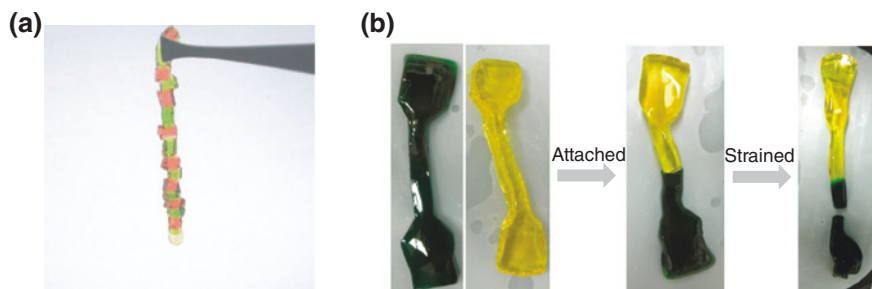
While stimuli-responsive polymers were designed to function as passive structures, there has also been a dynamic growth in interest in dynamically restructuring or self-healing polymers in recent years [240]. Such materials can undergo autonomic healing to repair damage and thus offer a new route towards safer, longer-lasting products and components. Among them, self-healing soft materials such as hydrogels are particularly promising for a variety of medical applications owing to their biocompatibility and mechanical similarity to natural tissues. Self-healing hydrogels, for example, have unique advantages as injectable biomaterials, which are increasingly being explored to minimize the risks and complications associated with surgical implantation [241]. The first reported approach for the design of self-healing materials was based on the microencapsulation of a reactive species that can polymerize or react with the matrix when released upon rupture of the encapsulating agents [242]. This reaction, at the damage site, was irreversible and the supply of healing agents was depleted locally. Therefore, the repair could not be repeated. Although some other approaches were adopted to attain multiple

**Fig. 2.19** Reversible molecular adsorption based on multiple-point interaction by shrinkable gels. Shrunken gels at 55 °C (*upper dish*) and swollen gels at 25 °C (*lower dish*) under illumination with UV. In the shrunken state, the gel adsorbed all of the pyranine molecules, but in the swollen state, the gel released them all, as shown by their fluorescence [239]



healing cycles of a single crack, repetitive healing was very rare in such systems. From these perspectives, intrinsic self-healing materials capable of repetitive repair are an increasingly active research area of particular scientific and commercial interest.

Intrinsic self-healing can be accomplished through thermally reversible reactions [243], hydrogen bonding [244], ionomeric coupling [245], a dispersed melttable thermoplastic phase [246], or polymer diffusion [247]. Among these strategies, the gelation techniques based on selective or specific interactions were found to be more promising. Host–guest interactions, for example, have been widely used in the construction of self-healing hydrogels (Fig. 2.20a). The combination of multiple noncovalent interactions, such as hydrogen bonding,  $\pi$ – $\pi$  stacking, charge transfer, and hydrophobic interactions between two complementary compounds, not only gives them a good binding affinity but also allows them to form complexes with fixed host–guest geometry and directionality [248]. On the other hand, metal–ligand interactions were also found to be promising because they are not only thermodynamically stable but also kinetically labile. Moreover, their reversibility can be selectively tuned by using different metal ions. Beck and Rowan have demonstrated the construction of room-temperature healable gels via the self-assembly of ditopic ligands, consisting of a 2, 6-bis(1-methylbenzimidazolyl) pyridine (BIP) moiety attached to either end of a PEG core, in the presence



**Fig. 2.20** Photographs of self-healing hydrogels. The self-healing is accomplished through **a** host–guest interactions [248], and **b** metal–ligand complexation [251]

of transition metal/lanthanide ions [249]. Holten-Andersen et al. [250] have developed a simple method for controlling catechol– $\text{Fe}^{3+}$  interpolymer crosslinking, inspired by the pH jump experienced by proteins during the maturation of a mussel byssus secretion. The catechol– $\text{Fe}^{3+}$  bonds can spontaneously reform after breaking, and such a network displays high elastic moduli. The authors have also prepared rapid self-healable and biocompatible hydrogels by the selective formation of metal–ligand complexes between selected metal ions and phosphate end groups of PEG (Fig. 2.20b). The gels were rapidly formed with trivalent metal ions such as  $\text{Fe}^{3+}$ ,  $\text{V}^{3+}$ ,  $\text{Al}^{3+}$ ,  $\text{Ti}^{3+}$ , and  $\text{Ga}^{3+}$ , which have small ion radii. We have also demonstrated a gel–sol/sol–gel transition by switching the redox states of  $\text{Fe}^{3+}/\text{Fe}^{2+}$  ions [251]. Learning from biological systems, the proposed phosphate–metal–ion–based self-healable hydrogels could become attractive candidates for various biomedical and environmental applications.

## 2.6 Conclusions and Future Trends

Smart hydrogels have the remarkable ability to respond to stimuli in a variety of ways. Because some environmental variables, such as low pH and elevated temperatures, are found in the body, smart hydrogels have enormous potential in various biomedical applications. For example, different types of smart hydrogels have been investigated for a series of drugs *in vitro* or *in vivo*. As a result, new and interesting controlled and sustained delivery strategies have become available. The fascinating properties of the stimuli-sensitive polymers are promising for many future applications and offer possible use as the next generation of materials in biological, biomedical and pharmaceutical products. Fundamental studies also greatly contributed to our present understanding of this unique class of materials. Although the concepts of these smart hydrogels are sound, the practical applications require significant improvements in the hydrogel properties. The most significant weakness of all these hydrogels is that their response time is too slow.

Thus, fast-acting hydrogels are still necessary. The synthesis of new polymers and cross linkers with more biocompatibility and better biodegradability would also be essential for successful applications. It is also expected that principles from the expanding research area of supramolecular chemistry will be applied to design novel types of hydrogels with tailored properties, which can preferably be prepared in an aqueous environment. Also, protein engineering might contribute to the development of hydrogel systems with very precise control over their microstructure, and thus, their properties. Again, smart hydrogels are an interesting class of materials that can be prepared by a variety of methods. Concurrent developments in the design of new responsive polymers along with structure-property evaluations are vital for practical applications of smart hydrogels. Not only does this enable the application of smart hydrogels to existing scientific problems, but it also allows previously unimagined technological directions to be explored.

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