Biomedical Hydrogels: Biochemistry, Manufacture and Medical Applications
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Hydrogel swelling behavior and its biomedical applications

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Abstract: The ability of hydrogels to respond to relatively small changes in stimuli with relatively large changes in volume allows a wide variety of applications. This chapter addresses hydrogels with regard to the chemical identity of hydrophilic polymers and copolymers, polymer synthesis, the degree of crosslinking and hydrogel porosity, and bulk geometry of hydrogels in the form of matrix, membrane and erodible systems. The relationships between these features and hydrogel swelling behavior upon stimulation are also described. Finally, various exploitations of hydrogel swelling behavior in developing highly sensitive, real-time biosensors are discussed.

Key words: hydrogels, superporous hydrogels (SPHs), swelling, environment-sensitive.

1.1 Basics of hydrogels

Hydrogels gained increased attention from the scientific community in the latter half of the 20th century (Brannon-Peppas and Peppas, 1991). The ability of hydrogels to respond to relatively small changes in external stimuli with relatively large changes in bulk volume enables direct detection of a variety of stimuli (Gemeinhart *et al*., 2000; Lee and Park, 1996; Peppas *et al*., 2000; Roy and Gupta, 2003). The chemical makeup, synthesis, crosslinking, and geometry of hydrogels are briefly described.

1.1.1 Chemical identity of hydrophilic polymers and copolymers

Hydrogels are composed of hydrophilic polymer chains. These chains may consist of repeating monomers (homopolymers) or chemically different monomers (copolymers) (Peppas *et al*., 2000). As depicted in Fig. 1.1, monomers can be arranged in such a way to make random copolymers, alternating copolymers, block copolymers, or graft copolymers. Additionally, the polymer chains may form more intricate three-dimensional structures, such as five-pointed star polymers, or dendrimers (Jeong *et al*., 2002). The selection of chemical makeup of a polymer is critical to controlling swelling behavior, since these constituents are responsible for interactions with water and subsequent volume change (Peppas *et al*., 2000). For example, hydrogels with hydrophobic internal cores would be well suited for delivery of poorly water-soluble drugs (Jeong *et al*., 2002).

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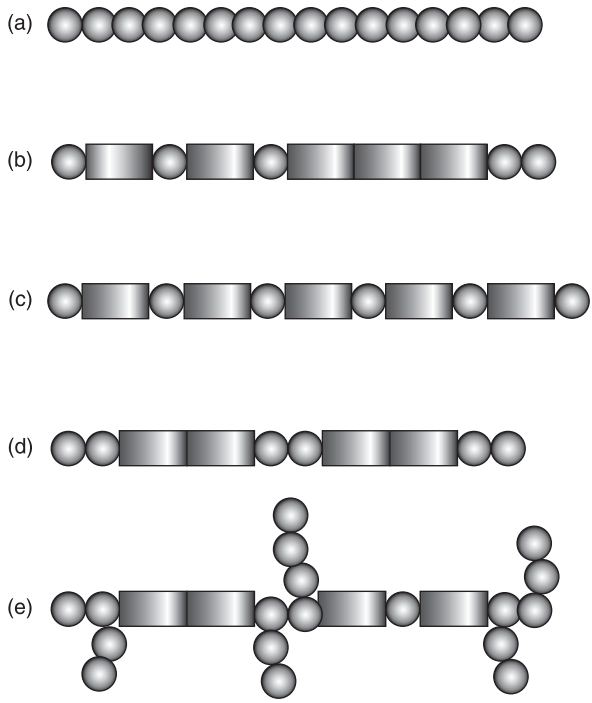

1.1 Chemical diversity of hydrogel polymer chains. (a) homopolymer, (b) random copolymer, (c) alternating copolymer, (d) block copolymer, and (e) graft copolymers. Rectangular and circular units represent chemically different monomers.

1.1.2 Polymer synthesis

Polymer synthesis is tailored according to the need to develop chemically diverse hydrogels for specific applications (Brannon-Peppas and Peppas, 1991; Peppas *et al*., 2000). Depending on the application, the synthesized polymers may require biocompatibility, mechanical strength, or analyte specificity in addition to sensitivity to stimuli (Brannon-Peppas and Peppas, 1991; Lee and Park, 1996; Peppas *et al*., 2000; Kim and Park, 2001b; Kim and Park, 2004). Table 1.1 lists monomers used in synthesizing hydrogels for pharmaceutical applications. Polymers are synthesized by various mechanisms, such as radical polymerization, condensation polymerization, graft-copolymerization, photopolymerization, and ring-opening polymerization (Lee and Park, 1996; Kim and Park, 2001b; Lee *et al*., 2003; Xiao, 2007; Pearton *et al*., 2008; Xue *et al*., 2004; Plunkett *et al*., 2003; Gu *et al*., 2002). Care must be taken to purify the synthesized hydrogels for pharmaceutical and biomedical applications by removing the residual monomer, initiator, crosslinking agent and other contaminants (Markowitz *et al*., 1997; Risbud *et al*., 2000; Peppas *et al*., 2000).

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Table 1.1 Examples of monomers used in pharmaceutical applications

Monomer
Acrylic acid (AA)
Ethylene glycol (EG)
Hydroxyethyl methacrylate (HEMA)
N-isopropyl acrylamide (NIPAAm)
N-vinyl-2-pyrrolidone (NVP)
Poly(ethylene glycol acrylate) (PEGA)

Source: Peppas *et al.* (2000)

1.1.3 Degree of crosslinking and porosity

Hydrogels are crosslinked either physically or chemically to form networks (Peppas *et al*., 2000; Roy and Gupta, 2003). Physical crosslinking occurs via noncovalent interactions, whereas chemical crosslinking utilizes covalent interactions (Lin *et al*., 2005). The degree of crosslinking plays a significant role in the integrity and swelling properties of hydrogels, influencing hydrogel structure and swelling capacity (Flory and Rehner, 1943; Brannon-Peppas and Peppas, 1991). The greater the extent of crosslinking, the less flexible a hydrogel is to shrink, swell or change phase in response to stimuli (Peppas *et al*., 2000). Hydrogel brittleness has been observed at high degrees of crosslinking (Peppas *et al*., 2000). Physical crosslinks are often used in hydrogel formation due to their ability to reform crosslinks upon removal or presentation of the stimulus (Roy and Gupta, 2003; Lee and Park, 1996; Lee *et al*., 2004).

Hydrogels have a range of porosities that influence the diffusion coefficients involved in mass transfer during swelling (Peppas *et al*., 2000; Bezemer *et al*., 2000). Pore size is dependent on the average molecular weight of polymer chain segments between adjacent crosslinks and acts as a selective barrier with regard to the permeability of substances (Peppas *et al*., 2000). Specifically, swelling can be decreased by decreasing the average molecular weight of the polymer chain segments between crosslinks (Brannon-Peppas and Peppas, 1991). Pore size can be further controlled by various techniques, such as freeze drying, porosigen method, or gas formation method (Gemeinhart *et al*., 2000). Therefore, the pores can range from a few nanometers to several micrometers (Kim and Park, 2004).

1.1.4 Bulk geometry of hydrogels

Hydrogels can be molded into various geometries, ranging from microspheres to films, and this makes their application highly versatile (Roy and Gupta, 2003). Hydrogel matrixes can be used as implantable scaffolds, due to their structural

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properties and ability to absorb or release bioactive substances (Roy and Gupta, 2003; Pearton *et al*., 2008; Markowitz *et al*., 1997; Risbud *et al*., 2000; Lee *et al*., 2003; Mauck *et al*., 2002; Gombotz and Wee, 1998). Table 1.2 lists hydrogels that have been studied for controlling the release of bioactive substances. Due to the relative thickness of a hydrogel matrix (as compared to membranes), the rate of diffusion for drug molecules through the matrix may be impeded (Zhang and Wu, 2002). Conversely, hydrogel membranes are relatively thin and offer increased response rate (swelling or shrinking) to stimuli due to the shorter distance required for diffusion (Zhang and Wu, 2002). Such hydrogels, capable of preventing degradation of labile substances, act as their reservoirs until stimulated (Pearton *et al*., 2008; Markowitz *et al*., 1997; Risbud *et al*., 2000; Mauck *et al*., 2002; Bezemer *et al*., 2000). Similarly, erodible hydrogels are of interest in the

Table 1.2 Examples of the use of hydrogels in biomedical applications

Hydrogel composition	Substance released	Stimulus	Ref.
Polyacrylic Carobopol-940 or PLGA-PEG-PLGA	Plasmid DNA (pDNA)	Hydration Temperature	(Pearton *et al.*, 2008)
Polyacrylamide	Monoclonal antimouse IgG-FITC	Hydration	(Markowitz *et al.*, 1997)
Poly(chitosan-pyrrolidone)			(Risbud *et al.*, 2000)
PEG-PLGA-PEG Agarose hydrogel	Plasmid TGF-β1 Chondrocytes*	Temperature	(Lee *et al.*, 2003) (Mauck *et al.*, 2002)
α-tocopheryl methacrylate-co-2-hydroxyethyl methacrylate (VEMA-co-HEMA)	α–tocopherol	pH	(Plasencia *et al.*, 1999)
Poly(HEMA-co-DMA), GOD	Insulin	Glucose	(Brahim *et al.*, 2002)
Poly(ethylene glycol)/poly (butylene terephthalate)	Lysozyme	Hydration	(Bezemer *et al.*, 2000)
Alginate microbeads	Albumin, HRP, Insulin, TGF-α, Hepatocytes		(Gombotz and Wee, 1998; Singh and Burgess, 1989; Igari *et al.*, 1990; Gray and Dowsett, 1988; Downs *et al.*, 1992; Miura *et al.*, 1986)

Note:* seeded in hydrogel

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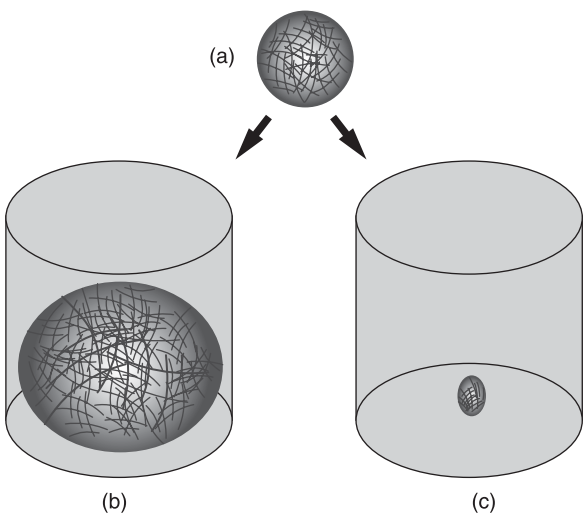
pharmaceutical field as their ability to exhibit zero-order release kinetics has been well established (Peppas *et al*., 2000; Lee, 1984).

1.2 Swelling of hydrogels: water diffusion into hydrogels

The ability to display a measurable change in volume in response to external stimuli is a fundamental property of hydrogels (Lee and Park, 1996). Some hydrogels exhibit this volume change by swelling (see Fig. 1.2), while others undergo transitions between sol and gel phases (Brannon-Peppas and Peppas, 1991; Gemeinhart *et al*., 2000; Lee and Park, 1996; Jeong *et al*., 2002). When hydrogels swell, the glassy phase turns into the rubbery phase (Lee, 1984). The degree of crosslinking influences the area permitted for diffusion across the hydrogel network and, subsequently, the capacity for hydrogels to take up water (Peppas *et al*., 2000). The water capacity is depicted from the equilibrium swelling ratio shown in Equation 1.1, as the ratio of the mass of a fully swollen hydrogel (in equilibrium with aqueous medium) to the mass of a dehydrated hydrogel (Brannon-Peppas and Peppas, 1991).

$$\text{Swelling ratio} = \frac{M_{\text{hydrated}} - M_{\text{dehydrated}}}{M_{\text{dehydrated}}} \qquad [1.1]$$

where M represents hydrogel mass. Interactions between polymers in hydrogels and water are similar in nature to those between non-crosslinked polymers and



1.2 Dehydrated (a), swollen (b), and shrunken (c) hydrogels as the result of small changes in external stimuli, such as pH, temperature and analyte concentration that influence hydrogel hydrophilicity.

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water. Hydrogels, consisting of networks of crosslinked hydrophilic polymers, undergo swelling instead of dissolution in water (Peppas *et al*., 2000). Hydrogels made of polyelectrolytes swell more due to the charge repulsion among polymer chains, and such swelling property is useful in environment-sensitive swelling of hydrogels for controlled drug release (Peppas *et al*., 2000; Roy and Gupta, 2003).

In the dehydrated state, hydrogels exist in the glassy state (Lee, 1984). The hydrogel may contain a substance of choice incorporated into the polymer network (Lee, 1984). As a glassy hydrogel swells, the inner portion of the hydrogel remains in a glassy phase, while the portion of the hydrogel that swells develops into a rubbery phase, expanding to accommodate water fluxed in (Peppas *et al*., 2000). Substances in the glassy phase are extremely slow in diffusing through the hydrogel network, while substances located in the outer, rubbery phase are released easily (Peppas *et al*., 2000). A swelling agent can be included to penetrate the hydrogel more rapidly than the encapsulated substance would normally diffuse, enhancing substance release through the swollen network (Lee, 1984; Peppas *et al*., 2000).

Hydrogels can have ionic or neutral side groups attached to their backbone chains, and either group will influence water uptake (Peppas *et al*., 2000). The Flory-Rehner theory aids in describing swelling of neutral hydrogels (Peppas *et al*., 2000). Briefly, a neutral hydrogel experiences a thermodynamic force of mixing and a contractive force that become balanced once a hydrogel reaches its equilibrium swelling state (Peppas *et al*., 2000). The theory was modified by Peppas and Merrill to account for hydrogels synthesized in water (Peppas *et al*., 2000). Anionic and cationic hydrogels have an additional force exerted on their networks due to their ability to form ionic interactions (Peppas *et al*., 2000). Peppas and Merrill derived relationships between ionic strength, the swollen state hydrogel volume fraction, and the average molecular weight of a polymer chain segment between two adjacent crosslinks, for polyelectrolyte hydrogels (Peppas *et al*., 2000). Altering the ionic strength of the hydrogel swelling agent (via salting out or salting in reagents) influences the equilibrium swelling volume (Peppas *et al*., 2000; Jeong *et al*., 2002; Suzuki and Kumagai, 2003).

Superporous hydrogels (SPHs) are capable of rapid swelling and shrinking via capillary forces (Gemeinhart *et al*., 2000). Fast swelling occurs as a result of convection of water into the porous hydrogels. Specifically, SPHs have pore sizes to the order of 10–1,000 μm, formed by gas blowing during hydrogel synthesis and gelation (Kim and Park, 2004). Because of the highly porous structure, SPHs often lack the mechanical strength required to be effective biosensors (Kim and Park, 2004). Polyethylenimine (PEI) interpenetrating polymer networks (IPNs) have been incorporated into poly(acrylamide-co-acrylic acid) P(AAm-co-AA) SPHs to improve the compressive strength (Kim and Park, 2004). PEI has a highly branched and ionizable structure capable of interacting

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with P(AAm-co-AA) electrostatically (Kim and Park, 2004). Reduced pore size from the resulting interactions with PEI was the trade-off for improved strength (Kim and Park, 2004).

1.3 Stimulus-responsive hydrogels

1.3.1 Linear hydrogel responses to external stimulus

Environment-sensitive hydrogels usually respond to external stimuli in a linear fashion after a stimulus reaches a setpoint. Hydrogels that can release insulin as a function of glucose concentration in the environment usually exhibit linear responses (Zhang and Wu, 2002; Kim and Park, 2001b; Obaidat and Park, 1997). Frequently, there is a lag time after the hydrogel is first stimulated until it responds to the stimulus (Kim and Park, 2001b; Obaidat and Park, 1997). If the hydrogel swelling depends on the changes in environment initiated by the components attached to the polymer chains (e.g., immobilized enzymes or other bioactive molecules), the hydrogel response rate is further delayed (Suzuki and Kumagai, 2003).

A hydrogel-actuated microvalve (HAM) was designed to respond to glucose concentration (Gu *et al*., 2002). Specifically, the HAM was a phenylboronic acid-based hydrogel, configured in an apparatus to modulate fluid flow, depending on whether the hydrogel was in a swollen or shrunken state (Gu *et al*., 2002). A swollen hydrogel closed the valve, while a shrunken hydrogel permitted flow (Gu *et al*., 2002). The HAM device had consistent responses (depicted as a flow rate) to changes in glucose concentration (Gu *et al*., 2002).

1.3.2 Hysteresis in hydrogel responses to external stimulus

Hydrogel swelling and response rate are further complicated when the stimulus setpoint is modulated as depicted in Fig. 1.3 (Zhang and Wu, 2002; Kim and Park, 2001b; Kataoka *et al*., 1998; Xiao, 2007; Satish and Shivakumar, 2007). The main challenges in development of environment-sensitive hydrogels are to make the hydrogels able to detect small changes in the stimulus with corresponding response, and, equally importantly, to maintain the hydrogel sensitivity over the entire spectrum of stimulus setpoints as well as during the lifetime of the intended applications. The ingenuity of future hydrogel developments will lie in the ability to predict and reproduce hydrogel swelling response with repetitive stimulation. Although hydrogels are capable of undergoing reversible transitions, repeated cycling between phases does not imply that mass transport through the hydrogel is reproducible on each cycle (Kim and Park, 2001b; Zhang and Wu, 2002; Miyata *et al*., 2002; Makino *et al*., 1990; Satish and Shivakumar, 2007). It is yet to be identified how the formation and re-formation of crosslinks during cycling account for altered mass transport in reversible hydrogel systems.

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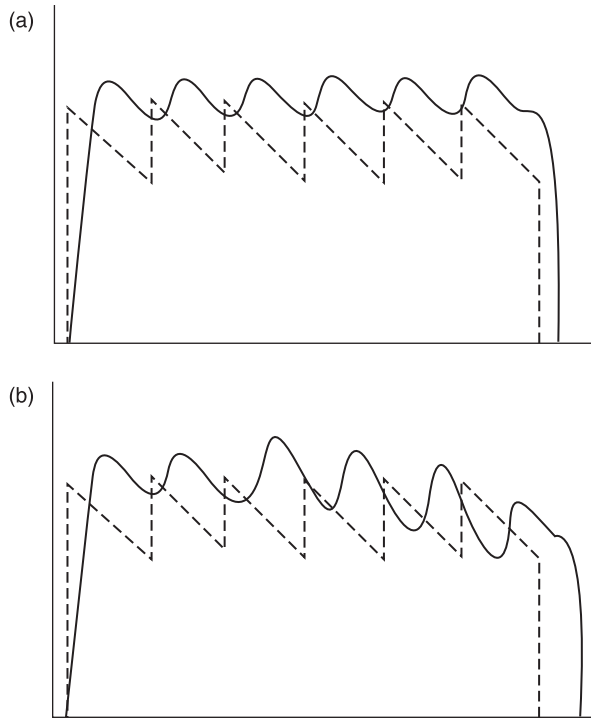

1.3 Modulation of external stimulus and subsequent hydrogel response. (a) theoretical hydrogel response to stimulus with respect to time. Constant hydrogel response rate and level baseline are achieved over time. (b) Hysteresis in hydrogel response to stimulus with respect to time. Hydrogel response rate and baseline level are time-dependent. Dashed grey line represents a change in stimulus setpoint with respect to time. Solid black line represents hydrogel response with respect to time.

Studies utilizing antigen-antibody sensitivity incorporated in a hydrogel network showed reproducible swelling behavior during a limited number of repeated cycles (Miyata *et al*., 1999; Miyata *et al*., 2002). Here, swelling resulted from introduction of free antigen to the hydrogel, which competes with the antigen attached to the hydrogel polymer chains (Miyata *et al*., 1999; Miyata *et al*., 2002).

One study employing a disposable hydrogel biosensor showed that hydrogel glucose-sensitivity changed with time (Suzuki and Kumagai, 2003). Cycling through stimulus setpoints has revealed an increase in hydrogel response rate over time in some hydrogel delivery systems (Kim and Park, 2001b; Zhang and Wu, 2002). Specifically, glucose-sensitive hydrogels, such as (poly(allyl glucose-co-3-sulfopropylacrylate) (P(AG-co-SPAK), poly(allyl glucose-co-N-vinyl pyrrolidone) (P(AG-co-VP), and poly(allyl glucose-co-acrylamide) (P(AG-co-AAm), exhibited faster release rates of insulin when exposed to cycles of either 1 or 4 mg/mL glucose solutions (Kim and Park, 2001b). Furthermore, the baseline for

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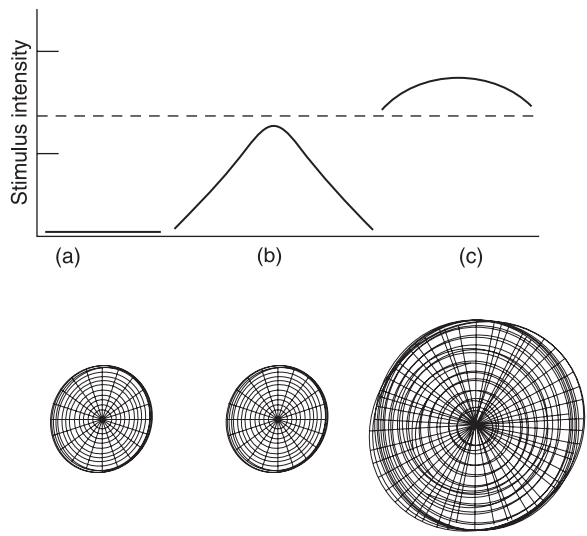
such responses rates drifted significantly upon cycling between predetermined setpoints, confirming that some inherent feature of the hydrogels was altered upon resuming the original setpoint (Kim and Park, 2001b; Kataoka *et al*., 1998; Xiao, 2007; Miyata *et al*., 2002; Makino *et al*., 1990; Satish and Shivakumar, 2007). Other hydrogel systems consisting of PNIPAAm exhibit constant response rates during cycling, yet fail to achieve reproducible swelling behavior over time (Xiao, 2007).

A likely reason for hysteresis in the response behavior is deterioration of the hydrogel components with repeated exposure to stimuli (Kim and Park, 2001b). Measures have been taken to reduce hydrogel degradation by enclosing hydrogels in dialysis tubes or membranes that allow the free flow of water and small solutes (Lee and Park, 1996; Obaidat and Park, 1997). Specifically, the inability of physical crosslinks to completely reform once dissociated may be responsible for the increased release rates observed. As a result of incomplete crosslink formation, pore size may remain enlarged, allowing diffusion of more drug molecules. Additionally, re-formation of some crosslinks may have a time-dependence. The chemical species involved in hydrogel stimulus recognition may also be affected during cycling.

1.3.3 Delayed swelling (threshold-dependent swelling)

Hydrogels can be designed to respond only beyond a certain threshold stimulus intensity but do not respond to stimuli although present below the threshold, as conveyed in Fig. 1.4 (Lee and Park, 1996; Kim and Park, 2001b; Kikuchi and



1.4 Overcoming a stimulus threshold to elicit a hydrogel response. Hydrogel behavior without stimulus (no swelling) (a), below stimulus threshold (no swelling) (b), and above stimulus threshold (swelling) (c). The X-axis represents different degrees of stimulation.

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Okano, 2002; Kataoka *et al*., 1998). An example of the threshold-dependent response is the use of the concanavalin A (Con A) lectin as a crosslink of glucose containing polymers (allyl glucose) (Lee and Park, 1996; Obaidat and Park, 1997; Kim and Park, 2001b). Soluble free glucose is supplied four times the molar quantity of Con A incorporated into the hydrogel structure in order to elicit a sol–gel phase response (Lee and Park, 1996). Free glucose has the ability to competitively bind with Con A, replacing some of the allyl glucose bound to Con A, and facilitating a sol–gel phase transition (Lee and Park, 1996). Reducing hydrogel dimensions and thickness may improve the hydrogel response rate (Obaidat and Park, 1997).

1.4 Examples of environment-sensitive hydrogels

Environment-sensitive hydrogels have the capability to imitate feedback mechanisms often observed in nature (Miyata *et al*., 2002). For instance, glucose, cholesterol and galactose amperometric hydrogel biosensors have been designed (Brahim *et al*., 2002). Impressively, it has been suggested that these sensors exhibit quick, linear responses to their respective stimuli (Brahim *et al*., 2002).

A variety of environment-sensitive hydrogels have been designed to harness the hydrogel swelling potential into a sensory device (see Table 1.3) (Roy and Gupta, 2003). The most common hydrogel systems swell in response to changing pH, temperature, and analyte concentration (Roy and Gupta, 2003).

1.4.1 pH-sensitive hydrogels

Hydrogels made of polyelectrolytes serve as pH-sensitive sensors (Peppas *et al*., 2000). Depending on solution pH and dissociation constants (pKa or pKb) of polymer side groups, the hydrogel becomes ionized and swells as a result of electrostatic repulsion of polymer chains (see Fig. 1.5) (Brannon-Peppas and Peppas, 1991; Peppas *et al*., 2000). Conversely, as the hydrogel becomes unionized, it shrinks due to reduced electrostatic repulsion (Peppas *et al*., 2000). For instance, N,O-carboxymethyl chitosan (NOCC) and alginate copolymer hydrogels were synthesized for use as carriers for oral administration of protein drugs (Mi *et al*., 2005). NOCC behaves as a zwitterion over a range of pH values,

Table 1.3 Examples of ion-sensitive natural hydrogels

Hydrogel composition	Stimulus	Ref.
Alginate	Ca^{2+} and other divalent ions	(Roy and Gupta, 2003; Gombotz and Wee, 1998; Byrom, 1991)
Chitosan	Mg^{2+} or pH	(Roy and Gupta, 2003)
κ-carrageenan	K^{+}	(Roy and Gupta, 2003)

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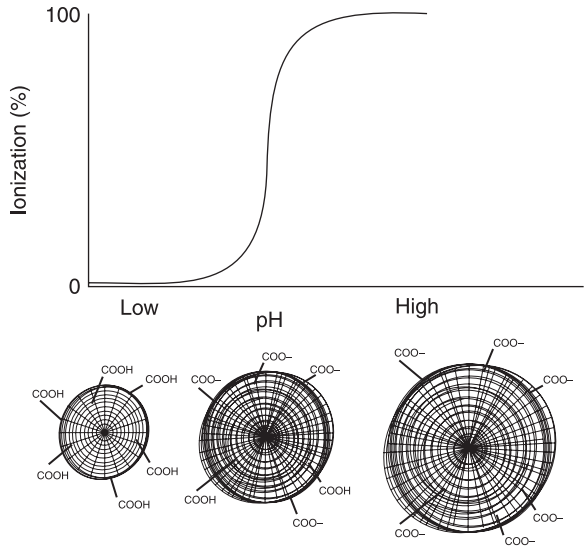

1.5 Swelling behavior of pH-sensitive (acidic) hydrogel. Acidic groups are unionized (bottom left), partially ionized (bottom middle), or completely ionized (bottom right).

and was crosslinked to alginate covalently using either genipin or glutaraldehyde, or ionically using calcium ions (Mi *et al*., 2005). The swelling ratios for the three types of crosslinked NOCC/alginate hydrogel systems were observed over acidic and slightly alkaline values at physiologic temperature in an attempt to simulate conditions in the gastrointestinal tract (Mi *et al*., 2005). Additionally, drug release was simulated from these three hydrogel types using 1% (w/v) bovine serum albumin (BSA) (Mi *et al*., 2005). It was observed that hydrogels consisting solely of NOCC had relatively high swelling ratios that decreased with the introduction of alginate during synthesis (Mi *et al*., 2005). Additionally, the lowest swelling ratios for the three crosslinked types of hydrogels all coincided at pH 4, suggesting low electrostatic interactions between NOCC and alginate (Mi *et al*., 2005).

In yet another pH-sensitive hydrogel system utilizing chitosan as a drug carrier to the colon, sodium tripolyphosphate (Na+-TPP) and dextran sulfate (DS) were incorporated into porous hydrogel microspheres (Lin *et al*., 2005). Specifically, chitosan, chitosan/Na^+-TPP and chitosan/Na^+-TPP/DS hydrogels were observed for their relative swelling properties and drug release (Lin *et al*., 2005). Ibuprofen was chosen to depict the ability of the microspheres to adequately encapsulate hydrophobic drugs in both alkaline and acidic media (Lin *et al*., 2005). In the hydrogel categories listed above, chitosan functioned as the polycation, while ionized Na^+-TPP and DS functioned as anions (Lin *et al*., 2005). In all hydrogel categories, swelling ratios increased with increasing pH, where most chitosan amines are deprotonated and negative charges of DS and TPP become dominant

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Table 1.4 Examples of pH-sensitive hydrogels

Hydrogel composition	Substance released	Stimulus	Ref.
Poly(acrylamide-co-acrylic acid)/ polyethylenimine interpenetrating network (P(AAm-co-AA)/PEI IPN)		pH	(Kim and Park, 2004)
Poly(acrylamide-co-acrylic acid) (P(AAm-co-AA))		pH	(Gemeinhart *et al.*, 2000)
Chitosan, chitosan/Na+-TPP, chitosan/Na+-TPP/DS	BSA	pH	(Lin *et al.*, 2005)

(Lin *et al*., 2005). As the DS constituent of the chitosan-based hydrogels increases, the degree of swelling increases as a result of the hydrophilicity of the sulfate group (Lin *et al*., 2005). A partial list of pH-sensitive hydrogels is shown in Table 1.4.

1.4.2 Temperature-sensitive and phase-reversible hydrogels

Phase-reversible hydrogels do not swell but rather have the ability to change solubility from a free flowing solution to a gel phase and vice versa (Jeong *et al*., 2002; Lee and Park, 1996). Sol–gel (reversible phase) hydrogels have been designed to respond to changes in pH, temperature and analyte concentration, in addition to other stimuli in as little as 5–30 minutes (Lee and Park, 1996; Obaidat and Park, 1997). For temperature-sensitive sol–gel hydrogels, the transition between the solution and gel phases occurs at the upper critical solution temperature (UCST) or the lower critical solution temperature (LCST) (Peppas *et al*., 2000). This temperature can be identified upon inverting a vessel containing the hydrogel, and noting the temperature at which the gel phase begins to flow or the solution phase becomes restricted to flow (Jeong *et al*., 2002). The falling ball method has also been described for determining the sol–gel transition (Yoshida *et al*., 1998). Table 1.5 highlights the LCST for commonly synthesized hydrogels. For example,

Table 1.5 Examples of LCST values of some hydrogels

Polymer	LCST (°C)
Poly(*N*-isopropylacrylamide) (NIPAAm)	~ 32
Poly(ethylene glycol) (PEG)	~ 120
Poly(vinyl alcohol) (PVA)	~ 125
Poly(vinyl pyrrolidone) (PVP)	~ 160
Methylcellulose (MC)	~ 80

Source: Jeong *et al.* (2002)

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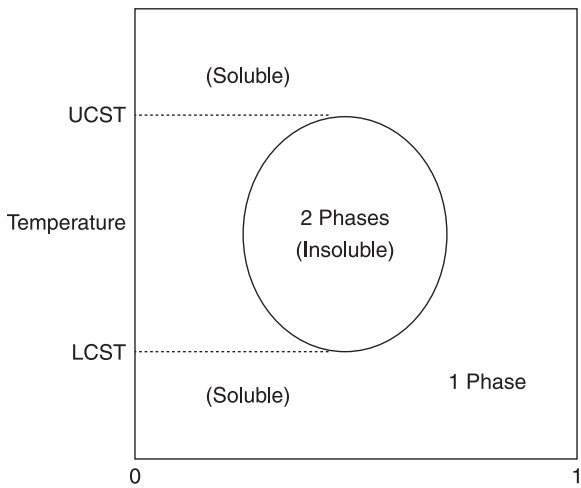
thermosensitive sol–gel hydrogels such as the poly(D,L-lactic-co-glycolic acid)-b-polyethylene glycol-b-poly(D,L-lactic-co-glycolic acid) (PLGA-PEG-PLGA) triblock copolymer forms a gel phase above the polymer's LCST (~ 32°C) (Pearton *et al*., 2008). A homogeneous solution phase exists below LCST, but a gel is formed or polymers precipitate at temperatures higher than LCST. If the temperature is increased further, the gel phase becomes a sol phase again. Poly(*N*-isopropylacrylamide) (PNIPAAm) has a similar LCST as PLGA-PEG-PLGA (Xiao, 2007). The presence of salting in or salting out reagents influences the observed transition temperature (Jeong *et al*., 2002; Suzuki and Kumagai, 2003; Kawasaki *et al*., 1997). Depending on the polymers used in synthesis, either a solution (T > UCST) or gel (LCST < T < UCST) phase may exist above the critical solution temperature, depicted in Fig. 1.6 (Peppas *et al*., 2000).

Other hydrogels are capable of undergoing a sol–gel phase transition in the excess of analyte concentration (Lee and Park, 1996; Jeong *et al*., 2002; Gemeinhart *et al*., 2000). In such circumstances, the stimulus is capable of inducing detachment of crosslinks, so that the hydrogel flows as a solution. Conversely, gelation occurs as crosslinks re-form among polymer chains. The ease of gel formation increases with increasing molar ratio of the crosslinking agent to backbone polymers (Lee and Park, 1996). Naturally occurring polymers that undergo sol–gel phase transition include chitosan (stimulated by pH), alginate (stimulated by calcium and other divalent ions excluding the magnesium ion), and



1.6 Phase changes of a temperature-sensitive polymer. At temperatures lower than LCST a homogeneous solution exists and a crosslinked hydrogel swells. As temperature increases above LCST, water-soluble polymer precipitates out of solution, and a crosslinked hydrogel shrinks.

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κ-carrageenan (stimulated by potassium ions) (Roy and Gupta, 2003; Gombotz and Wee, 1998; Byrom, 1991). Although these polymers are natural, their derivatives may contain impurities that may prove toxic if implanted directly *in vivo* (Gombotz and Wee, 1998). For instance, heavy metals, mitogens and endotoxins may exist in the kelp sources used in producing alginate (Gombotz and Wee, 1998; Smidsrod, 1973).

1.4.3 Glucose-sensitive hydrogels

Interest in glucose-sensitive hydrogels has increased over the last few decades, as the projected number of people (diagnosed and undiagnosed) living with diabetes increases (Boyle *et al*., 2001; Fagot-Campagna *et al*., 2000). A decade ago, it was suggested that a third of people living with non-insulin dependent diabetes-mellitus (NIDDM or type II diabetes) did not even realize that they had this disease (Engelgau *et al*., 1998; Petersen *et al*., 2003; Anon., 1997; Gavin *et al*., 1997). In addition to the disease itself, secondary diseases are further exasperated by NIDDM and insulin-dependent diabetes-mellitus (IDDM or type I diabetes) such as retinopathy, nephropathy, neuropathy and macrovascular disease (Control *et al*., 1993). Additionally it has been suggested that between 1988 and 1994, approximately 71% of adult diabetics in the U.S. had hypertension or prehypertension as well (average blood pressure ≥ 130/85 mm Hg) (Geiss *et al*., 2002). Prevention of these diseases and further damage to the body is dependent on the regulation of blood glucose (Control *et al*., 1993). The focus has frequently been on implantable devices that could sense abnormal glucose levels (generally hyperglycemic) in the body, and respond by delivering the appropriate amount of insulin, which signals glucose uptake into neighboring cells (Lee and Park, 1996; Obaidat and Park, 1997; Kim and Park, 2001b; Zhang and Wu, 2002; Zhang *et al*., 2007; Shenkman *et al*., 2007).

Concanavalin A and allyl glucose hydrogel systems

Glucose-sensitive hydrogel systems have been developed using concanavalin A (Con A), isolated from the *Canavalia ensiformis* jackbean, as a crosslinking agent of glucose-containing polymeric chains (allyl glucose), depicted in Table 1.6 (Kim and Park, 2001a; Kim and Park, 2001b; Lee and Park, 1996). As a tetramer at physiologic pH, the Con A lectin is capable of noncovalently binding four glucose molecules (Lee and Park, 1996; Kim and Park, 2001a; Kim and Park, 2001b). To maintain its structure and binding ability, seven co-ordinated calcium ions and six co-ordinated manganese (II) ions bind with Con A (Hardman *et al*., 1982). The hydroxyl groups on carbons three through six of the allyl glucose interact with Con A (Kim and Park, 2001a). In one study, the allyl attachment to glucose was made at carbon one (Kim and Park, 2001a). Below the threshold free glucose concentration, four allyl glucose chains are bound to Con A (Kim and

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Table 1.6 Examples of glucose-sensitive hydrogels

Hydrogel composition	Substance released	Stimulus	Ref.
1-vinyl-2-pyrrolidinone-allyl glucose (VP-AG),Con A		Glucose	(Lee and Park, 1996)
Poly(hydroxyethyl methacrylate) (PHEMA), Con A	Insulin, Lysozyme	Glucose	(Obaidat and Park, 1997)
Poly(allyl glucose-co-3-sulfopropylacrylate potassium salt) (P(AG-co-SPAK)), Poly(allyl glucose-co-vinyl pyrrolidone) (P(AG-co-VP)), Poly(allyl glucose-co-acrylamide) (P(AG-co-AAm)), Pegylated Con A	Insulin	Glucose	(Kim and Park, 2001b)
Poly(*N*-isopropylacrylamide-co-methacrylic acid) (P(NIPAAm-co-MAA)), Glucose oxidase (GOD), Catalase	Insulin	Glucose	(Zhang and Wu, 2002)
Poly(acrylamide-co-3-acrylamidophenylboronic acid) (P(AAm-co-3-AAmPBA))		Glucose, Cis-diols	(Lee *et al.*, 2004)
Poly(*N*-isopropylacrylamide) (PNIPAAm) core, Poly(*N*-isopropylacrylamide-co-phenylboronic acid) (PNIPAAm-co-PBA) shell		Glucose	(Zhang *et al.*, 2007)
Hydrogel matrix, Glucose binding protein (GBP), Cyan fluorescent protein (CFP), Yellow fluorescent protein (YFP)		Light (400–400 nm), Glucose	(Shenkman *et al.*, 2007)
Poly(2-hydroxyethyl methacrylate-co-*N, N*-dimethylaminoethyl methacrylate), Glucose oxidase (GOD), Catalase	Insulin	Glucose	(Satish and Shivakumar, 2007)

Park, 2001b). Specifically, allyl glucose has a higher binding affinity for Con A than free glucose below this threshold free glucose concentration (Kim and Park, 2001b). As the concentration of free glucose is increased to four times the concentration of Con A, free glucose begins to compete with allyl glucose for binding sites on Con A (Kim and Park, 2001a; Kim and Park, 2001b). Excess free glucose concentration induces a sol phase, whereas lower glucose concentration induces a gel phase (Kim and Park, 2001b). A major concern about the use of Con A as a constituent in implantable hydrogels is the immunogenicity associated with the lectin (Kim and Park, 2001a; Kataoka *et al*., 1998). To reduce immunogenicity and increase stability, poly(ethylene glycol) (PEG) units were grafted to Con A (Kim and Park, 2001a). A ratio of five PEG units to one Con A has displayed an optimum binding affinity of free glucose to Con A (Kim and Park, 2001b).

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Coupled glucose oxidase and catalase hydrogel systems

Researchers have looked towards 'natural' ways of implementing blood glucose control by encapsulating glucose oxidase (GOD) isolated from *Aspergillus niger* within a hydrogel network made of pH-sensitive polymers (Zhang and Wu, 2002; Satish and Shivakumar, 2007). Free glucose, presumably from the blood, can be converted to gluconic acid via GOD (Zhang and Wu, 2002; Satish and Shivakumar, 2007).

$$\text{Glucose} + O_2 + H_2O \rightarrow \text{gluconic acid} + H_2O_2 \qquad [1.2]$$

As the concentration of gluconic acid increases, the pH correspondingly decreases, causing the hydrogel to swell or shrink in response and releasing stored insulin to combat an increasing glucose concentration (Zhang and Wu, 2002; Satish and Shivakumar, 2007). Limitations in the use of immobilized GOD include the need to replenish depleted enzymes, and buildup of hydrogen peroxide as a result of reduction-oxidation reactions (Zhang and Wu, 2002; Satish and Shivakumar, 2007). Hydrogen peroxide, a product of GOD, inhibits GOD function (Zhang and Wu, 2002; Satish and Shivakumar, 2007). To counteract this inhibition, catalase isolated from *Aspergillus niger* has been coupled with GOD in hydrogel systems, to convert hydrogen peroxide back to free oxygen and water (Zhang and Wu, 2002).

$$H_2O_2 \rightarrow \frac{1}{2} O_2 + H_2O \qquad [1.3]$$

It has been reported that by incorporating catalase into the GOD immobilized hydrogel, as much as 50% of the oxygen used in converting glucose to gluconic acid can be recovered (Zhang and Wu, 2002). Additionally, it has been suggested that gluconolactonase may be used to increase the rate that gluconic acid is formed, converting the gluconolactone intermediate to gluconic acid (Suzuki and Kumagai, 2003; Hanazato *et al*., 1988; Ogawa *et al*., 2002). See Table 1.6 for a compilation of hydrogel systems utilizing GOD in the presence or lack of catalase.

Glucose binding protein and fluorescent resonance electron transfer (FRET) technology hydrogel systems for glucose sensing

A glucose binding protein (GBP) isolated from *Escherichia coli* was engineered and encapsulated in a hydrogel system (Shenkman *et al*., 2007). GBP was engineered to depend on two additional proteins, cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP), which act as an electron donor and acceptor, respectively (Shenkman *et al*., 2007). Initially stimulated by blue light (400–400 nm), CFP and YFP form a closed circuit in the absence of free glucose (Shenkman *et al*., 2007); thus, fluorescent resonance electron transfer (FRET) fluorescence from YFP is observed (Shenkman *et al*., 2007). In the presence of free glucose, a conformational change takes place in GBP, increasing the distance between CFP

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and YFP so that CFP is unable to donate its electron to YFP; therefore, fluorescence from CFP is observed (Shenkman *et al*., 2007). Using FRET, the concentration of free glucose in the hydrogel environment can be readily quantified (Shenkman *et al*., 2007).

Boronic acids and free glucose hydrogel systems

A glucose-sensitive hydrogel system made of boronic acid does not rely on lectins, enzymes or other proteins in detecting changes in glucose concentration (Kataoka *et al*., 1998). For example, phenylboronic acid has been frequently incorporated into hydrogels due to its ability to bind with free glucose (Kataoka *et al*., 1998). Specifically, polymer chains consisting of 3-acrylamidophenylboronic acid (3-AAmPBA) and poly(*N*-isopropylacrylamide) (PNIPAAm) become ionized in an alkaline environment (Kataoka *et al*., 1998). Ionization of phenylboronic acid encourages covalent, yet reversible binding with glucose (Kataoka *et al*., 1998; Lee *et al*., 2004). In a medium with pH > 9, the phenylboronic acid portions of the hydrogel partially ionize (Kataoka *et al*., 1998). As the concentration of glucose increases in the alkaline medium (pH 9), equilibrium shifts in favor of the ionized form of phenylboronic acid, which now has the ability to bind with glucose (Kataoka *et al*., 1998). As a result, increasing aqueous glucose concentration increases phenylboronic acid ionization, which in turn increases the repulsive charges on polymer chains (Kataoka *et al*., 1998). These charges increase hydrogel hydrophilicity and swelling is observed (Kataoka *et al*., 1998).

In yet another glucose-sensitive hydrogel involving 3-AAmPBA, an acrylamide hydrogel film containing a hologram has been developed with the ability to detect glucose as well as other cis-diols in the environment (Lee *et al*., 2004). Hydrogels of this sort would be useful nutrient monitors in bioreactors (Lee *et al*., 2004). Specifically, in the presence of alkaline medium, 3-AAmPBA groups of the hydrogel bind cis-diols (i.e. deoxyribose, fructose, galactose, lactate, glucose, etc.), resulting in an observed swelling of the hydrogel (Lee *et al*., 2004). Impinged with white light, the degree of hydrogel swelling shifts the light diffracted from the hydrogel from blue to red, depending on the cis-diol concentration (Lee *et al*., 2004). Holographic hydrogel response rate was faster for 2 mM lactate than for 2 mM glucose solutions, yielding half-lives of 0.7 min and 10.5 min, respectively (Lee *et al*., 2004).

1.5 Future trends

For clinical applications, future hydrogel biosensors will have to overcome several obstacles (Brahim *et al*., 2002). For instance, sensors should be resistant to biofouling if they are to be employed in implantation or if interactions with biological fluids are required (Brahim *et al*., 2002; Wisniewski and Reichert, 2000). While sufficiently sensitive and responsive to the specific stimuli, the

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'smart' biosensors should remain insensitive to commonly observed interferents such as acetaminophen, uric acid, and L-ascorbic acid (Brahim *et al*., 2002; Suzuki and Kumagai, 2003). Additionally, hydrogel biosensor sensitivity and stability over prolonged time and when not in use will need to be addressed (Brahim *et al*., 2002). Disposable biosensors may be an alternative for sensors lacking sufficient stability or mechanical strength over time (Suzuki and Kumagai, 2003). Additionally, in order to lower hydrogel detection limits for reasonable use, synthesis of smaller 'microgels' will need to be employed (Plunkett *et al*., 2003).

If the above challenges are overcome, hydrogel use may even prove convenient in diagnostic testing. It has been suggested that hydrogels, designed to be degraded by enzymes concomitant with specific disease states, can be used to determine the progression of disease states (Miyata *et al*., 2002; Plunkett *et al*., 2003). Targeted drug delivery may be enhanced with the use of hydrogels that degrade in the presence of specific enzymes. For instance, hydrogels have been synthesized to degrade with either dextranase or azoreductase, both which are readily available in the colon, for local treatment of diseases present in the colon (Hovgaard and Brondsted, 1995; Yeh *et al*., 1995). Others have synthesized hydrogels that require multiple stimuli to degrade hydrogel networks (Yamamoto *et al*., 1996; Kurisawa *et al*., 1997).

The subject of hydrogels has already allowed the integration of a variety of scientific backgrounds, in understanding both chemical functions of natural and synthetic materials and biological functions of small molecules (Miyata *et al*., 2002). For now, the ingenuity of future hydrogel developments will lie in the ability to predict and reproduce hydrogel swelling response with repetitive stimulation. For hydrogels to be used as real-time biosensors in the future, we must understand how the formation and re-formation of crosslinks during repetitive stimulation account for altered mass transport in phase reversible hydrogel systems.

1.6 References

Anon. (1997), 'National diabetes awareness month – November 1997', *Jama – Journal of the American Medical Association*, 278, 1564.

Bezemer JM, Radersma R, Grijpma DW, Dijkstra PJ, Feijen J and van Blitterswijk CA (2000), 'Zero-order release of lysozyme from poly(ethylene glycol) poly(butylene terephthalate) matrices', *Journal of Controlled Release*, 64, 179–92.

Boyle J, Honeycutt A, Narayan K, Hoerger T, Geiss L, Chen H and Thompson T (2001), 'Projection of diabetes burden through 2050 – Impact of changing demography and disease prevalence in the US', *Diabetes Care*, 24, 1936–40.

Brahim S, Narinesingh D and Guiseppi-Elie A (2002), 'Bio-smart hydrogels: co-joined molecular recognition and signal transduction in biosensor fabrication and drug delivery', *Biosensors and Bioelectronics*, 17, 973–81.

Brannon-Peppas L and Peppas N (1991), 'Equilibrium swelling behavior of pH-sensitive hydrogels', *Chemical Engineering Science*, 46, 715–22.

© Woodhead Publishing Limited, 2011

Byrom D (ed.) (1991), *Alginates in Biomaterials: Novel Materials from Biological Sources*, New York, Stockton Press.

Control TD and Complications Trial Research Group (1993), 'The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus', *New England Journal of Medicine*, 329, 977–86.

Downs EC, Robertson NE, Riss TL and Plunkett ML (1992), 'Calcium alginate beads as a slow-release system for delivering angiogenic molecules *in vivo* and *in vitro*', *Journal of Cellular Physiology*, 152, 422–9.

Engelgau M, Narayan K and Thompson J *et al*. (1998), 'The cost-effectiveness of screening for type 2 diabetes', *Journal of the American Medical Association*, 280, 1757–63.

Fagot-Campagna A, Pettitt D, Engelgau M, Burrows N, Geiss L, Valdez R, Beckles G, Saaddine J, Gregg E, Williamson D and Narayan K (2000), 'Type 2 diabetes among North American children and adolescents: An epidemiologic review and a public health perspective', *The Journal of Pediatrics*, 136, 664–72.

Flory P and Rehner, Jr. J (1943), 'Statistical mechanics of crosslinked polymer networks II swelling', *The Journal of Chemical Physics*, 11, 521–6.

Gavin JR, Alberti, *et al*. (1997), 'Report of the expert committee on the diagnosis and classification of diabetes mellitus', *Diabetes Care*, 20, 1183–97.

Geiss L, Rolka D and Engelgau M (2002), 'Elevated blood pressure among US adults with diabetes, 1988–1994', *American Journal of Preventive Medicine*, 22, 42–8.

Gemeinhart RA, Chen J, Park H and Park K (2000), 'pH-sensitivity of fast responsive superporous hydrogels', *Journal of Biomaterials Science-Polymer Edition*, 11, 1371–80.

Gombotz WR and Wee SF (1998), 'Protein release from alginate matrices', *Advanced Drug Delivery Reviews*, 31, 267–85.

Gray CJ and Dowsett J (1988), 'Retention of insulin in alginate gel beads', *Biotechnology and Bioengineering*, 31, 607–12.

Gu YD, Baldi A, Ziaie B and Siegel RA (2002), *Modulation of Drug Delivery Rate by Hydrogel-incorporating MEMS Devices*, New York, IEEE.

Hanazato Y, Inatomi K, Nakako M, Shiono S and Maeda M (1988), 'Glucose-sensitive field-effect transistor with a membrane containing co-immobilized gluconolactonase and glucose-oxidase', *Analytica Chimica Acta*, 212, 49–59.

Hardman K, Agarwal R and Freiser M (1982), 'Manganese and calcium-binding sites of concanavalin-A', *Journal of Molecular Biology*, 157, 69–86.

Hovgaard L and Brondsted H (1995), 'Dextran hydrogels for colon-specific drug-delivery', *Journal of Controlled Release*, 36, 159–66.

Igari Y, Kibat PG and Langer R (1990), 'Optimization of a microencapsulated liposome system for enzymatically controlled release of macromolecules', *Journal of Controlled Release*, 14, 263–7.

Jeong B, Kim S and Bae Y (2002), 'Thermosensitive sol-gel reversible hydrogels', *Advanced Drug Delivery Reviews*, 54, 37–51.

Kataoka K, Miyazaki H, Bunya M, Okano T and Sakurai Y (1998), 'Totally synthetic polymer gels responding to external glucose concentration: Their preparation and application to on-off regulation of insulin release', *Journal of the American Chemical Society*, 120, 12694–5.

Kawasaki H, Sasaki S and Maeda H (1997), 'Effect of introduced electric charge on the volume phase transition of N-isopropylacrylamide gels', *Journal of Physical Chemistry B*, 101, 4184–7.

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Kikuchi A and Okano T (2002), 'Pulsatile drug release control using hydrogels', *Advanced Drug Delivery Reviews*, 54, 53–77.

Kim D and Park K (2004), 'Swelling and mechanical properties of superporous hydrogels of poly (acrylamide-co-acrylic acid)/polyethylenimine interpenetrating polymer networks', *Polymer*, 45, 189–96.

Kim J and Park K (2001a), 'Glucose-binding property of pegylated concanavalin A', *Pharmaceutical Research*, 18, 794–9.

Kim J and Park K (2001b), 'Modulated insulin delivery from glucose-sensitive hydrogel dosage forms', *Journal of Controlled Release*, 77, 3947.

Kurisawa M, Terano M and Yui N (1997), 'Double-stimuli-responsive degradation of hydrogels consisting of oligopeptide-terminated poly(ethylene glycol) and dextran with an interpenetrating polymer network', *Journal of Biomaterials Science – Polymer Edition*, 8, 691–708.

Lee M, Kabilan S, Hussain A, Yang X, Blyth J and Lowe C (2004), 'Glucose-sensitive holographic sensors for monitoring bacterial growth', *Analytical Chemistry*, 76, 5748–55.

Lee P (1984), 'Effect of non-uniform initial drug concentration distribution on the kinetics of drug release from glassy hydrogel matrices', *Polymer*, 25, 973–8.

Lee P, Li Z and Huang L (2003), 'Thermosensitive hydrogel as a Tgf-beta 1 gene delivery vehicle enhances diabetic wound healing', *Pharmaceutical Research*, 20, 1995–2000.

Lee S and Park K (1996), 'Synthesis and characterization of sol-gel phase-reversible hydrogels sensitive to glucose', *Journal of Molecular Recognition*, 9, 549–57.

Lin W, Yu D and Yang M (2005), 'pH-sensitive polyelectrolyte complex gel microspheres composed of chitosan/sodium tripolyphosphate/dextran sulfate: swelling kinetics and drug delivery properties', *Colloids and Surfaces B: Biointerfaces*, 44, 143–51.

Makino K, Mack EJ, Okano T and Kim SW (1990), 'A microcapsule self-regulating delivery system for insulin', *Journal of Controlled Release*, 12, 235–9.

Markowitz M, Turner D, Martin B and Gaber B (1997), 'Diffusion and transfer of antibody proteins from a sugar-based hydrogel', *Applied Biochemistry and Biotechnology*, 68, 57–68.

Mauck R, Seyhan S, Ateshian G and Hung C (2002), 'Influence of seeding density and dynamic deformational loading on the developing structure/function relationships of chondrocyte-seeded agarose hydrogels', *Annals of Biomedical Engineering*, 30, 1046–56.

Mi F, Liang H, Wu Y, Lin Y, Yang T and Sung H (2005), 'pH-sensitive behavior of two-component hydrogels composed of N,O-carboxymethyl chitosan and alginate', *Journal of Biomaterials Science: Polymer Ed.*, 16, 1333–45.

Miura Y, Akimoto T, Kanazawa H and Yagi K (1986), 'Synthesis and secretion of protein by hepatocytes entrapped within calcium alginate', *Artificial Organs*, 10, 460–5.

Miyata T, Asami N and Uragami T (1999), 'A reversibly antigen-responsive hydrogel', *Nature*, 399, 766–9.

Miyata T, Uragami T and Nakamae K (2002), 'Biomolecule-sensitive hydrogels', *Advanced Drug Delivery Reviews*, 54, 79–98.

Obaidat A and Park K (1997), 'Characterization of protein release through glucose-sensitive hydrogel membranes', *Biomaterials*, 18, 801–6.

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Ogawa K, Nakajima-Kambe T, Nakahara T and Kokufuta E (2002), 'Coimmobilization of gluconolactonase with glucose oxidase for improvement in kinetic property of enzymatically induced volume collapse in ionic gels', *Biomacromolecules*, 3, 625–31.

Pearton M, Allender C, Brain K, Anstey A, Gateley C, Wilke N, Morrissey A and Birchall J (2008), 'Gene delivery to the epidermal cells of human skin explants using microfabricated microneedles and hydrogel formulations', *Pharmaceutical Research*, 25, 407–16.

Peppas N, Bures P, Leobandung W and Ichikawa H (2000), 'Hydrogels in pharmaceutical formulations', *European Journal of Pharmaceutics and Biopharmaceutics*, 50, 27–46.

Petersen M and American Diabetes Association (2003), 'Economic costs of diabetes in the US in 2002', *Diabetes Care*, 26, 917–32.

Plasencia MA, Ortiz C, Vazquez B, Roman JS, Lopez-Bravo A and Lopez-Alonso A (1999), 'Resorbable polyacrylic hydrogels derived from vitamin E and their application in the healing of tendons', *Journal of Materials Science – Materials in Medicine*, 10, 641–8.

Plunkett KN, Kraft ML, Yu Q and Moore JS (2003), 'Swelling kinetics of disulfide cross-linked microgels', *Macromolecules*, 36, 3960–6.

Risbud M, Hardikar A and Bhonde R (2000), 'Growth modulation of fibroblasts by chitosan-polyvinyl pyrrolidone hydrogel: Implications for wound management?', *Journal of Biosciences*, 25, 25–31.

Roy I and Gupta M (2003), 'Smart polymeric materials: Emerging biochemical applications', *Chemistry & Biology*, 10, 1161–71.

Satish CS and Shivakumar HG (2007), 'Formulation and evaluation of self-regulated insulin delivery system based on poly(HEMA-co-DMAEMA) hydrogels', *Journal of Macromolecular Science Part A – Pure and Applied Chemistry*, 44, 379–87.

Schild HG (1992), 'Poly(N-isopropylacrylamide) – Experiment, theory and application', *Progress in Polymer Science*, 17, 163–249.

Shenkman L, Koukaki M, Karamanou S and Economou A (2007), 'The P. Cezanne project: Innovative approaches to continuous glucose monitoring', *2007 Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, 1–16, 6061–4.

Singh ON and Burgess DJ (1989), 'Characterization of albumin-alginic acid complex coacervation', *Journal of Pharmacy and Pharmacology*, 41, 670–3.

Smidsrod O (1973), 'Relative extension of alginates having different chemical composition', *Carbohydrate Research*, 27, 107–18.

Suzuki H and Kumagai A (2003), 'A disposable biosensor employing a glucose-sensitive biochemomechanical gel', *Biosensors and Bioelectronics*, 18, 1289–97.

Wisniewski N and Reichert M (2000), 'Methods for reducing biosensor membrane biofouling', *Colloids and Surfaces B- Biointerfaces*, 18, 197–219.

Xiao X (2007), 'Effect of the initiator on thermosensitive rate of poly(N-isopropylacrylamide) hydrogels', *Express Polymer Letters*, 1, 232–5.

Xue W, Champ S, Huglin MB and Jones TGJ (2004), 'Rapid swelling and deswelling in cryogels of crosslinked poly(N-isopropylacrylamide-co-acrylic acid)', *European Polymer Journal*, 40, 467–76.

Yamamoto N, Kurisawa M and Yui N (1996), 'Double-stimuli-responsive degradable hydrogels: Interpenetrating polymer networks consisting of gelatin and dextran with different phase separation', *Macromolecular Rapid Communications*, 17, 313–18.

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Yeh PY, Kopeckova P and Kopecek J (1995), 'Degradability of hydrogels containing azoaromatic cross-links', *Macromolecular Chemistry and Physics*, 196, 2183–202.

Yoshida T, Takahashi M, Hatakeyama T and Hatakeyama H (1998), 'Annealing induced gelation of xanthan/water systems', *Polymer*, 39, 1119–22.

Zhang K and Wu X (2002), 'Modulated insulin permeation across a glucose-sensitive polymeric composite membrane', *Journal of Controlled Release*, 80, 169–78.

Zhang Y, Guan Y and Zhou S (2007), 'Permeability control of glucose-sensitive nanoshells', *Biomacromolecules*, 8, 3842–7.

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