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).
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).
Hydrogel swelling behavior and its biomedical applications

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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Table 1.1 Examples of monomers used in pharmaceutical applications

<table>
<thead>
<tr>
<th>Monomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylic acid (AA)</td>
</tr>
<tr>
<td>Ethylene glycol (EG)</td>
</tr>
<tr>
<td>Hydroxyethyl methacrylate (HEMA)</td>
</tr>
<tr>
<td>N-isopropyl acrylamide (NIPAAm)</td>
</tr>
<tr>
<td>N-vinyl-2-pyrrolidone (NVP)</td>
</tr>
<tr>
<td>Poly(ethylene glycol acrylate) (PEGA)</td>
</tr>
</tbody>
</table>

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

Note:* seeded in hydrogel
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).

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

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

![Diagram of hydrogels: (a) dehydrated, (b) swollen, (c) shrunken](image)

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.
Biomedical hydrogels

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.
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
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

![Diagram](image.png)

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.
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>
<thead>
<tr>
<th>Hydrogel composition</th>
<th>Stimulus</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginate</td>
<td>Ca(^{2+}) and other divalent ions</td>
<td>(Roy and Gupta, 2003; Gombotz and Wee, 1998; Byrom, 1991)</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Mg(^{2+}) or pH</td>
<td>(Roy and Gupta, 2003)</td>
</tr>
<tr>
<td>κ-carrageenan</td>
<td>K(^+)</td>
<td>(Roy and Gupta, 2003)</td>
</tr>
</tbody>
</table>
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.
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 1.4 Examples of pH-sensitive hydrogels

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

(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).

#### Table 1.5 Examples of LCST values of some hydrogels

<table>
<thead>
<tr>
<th>Polymer</th>
<th>LCST (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(N-isopropylacrylamide) (NIPAAm)</td>
<td>~ 32</td>
</tr>
<tr>
<td>Poly(ethylene glycol) (PEG)</td>
<td>~ 120</td>
</tr>
<tr>
<td>Poly(vinyl alcohol) (PVA)</td>
<td>~ 125</td>
</tr>
<tr>
<td>Poly(vinyl pyrrolidone) (PVP)</td>
<td>~ 160</td>
</tr>
<tr>
<td>Methylcellulose (MC)</td>
<td>~ 80</td>
</tr>
</tbody>
</table>

Source: Jeong et al. (2002)
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

![Diagram](image)

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.
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
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).

### Table 1.6 Examples of glucose-sensitive hydrogels

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

\[
\text{Glucose} + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{gluconic acid} + \text{H}_2\text{O}_2
\]  

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).

\[
\text{H}_2\text{O}_2 \rightarrow \frac{1}{2} \text{O}_2 + \text{H}_2\text{O}
\]

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
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


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Hydrogel swelling behavior and its biomedical applications


Biomedical hydrogels


Hydrogel swelling behavior and its biomedical applications


