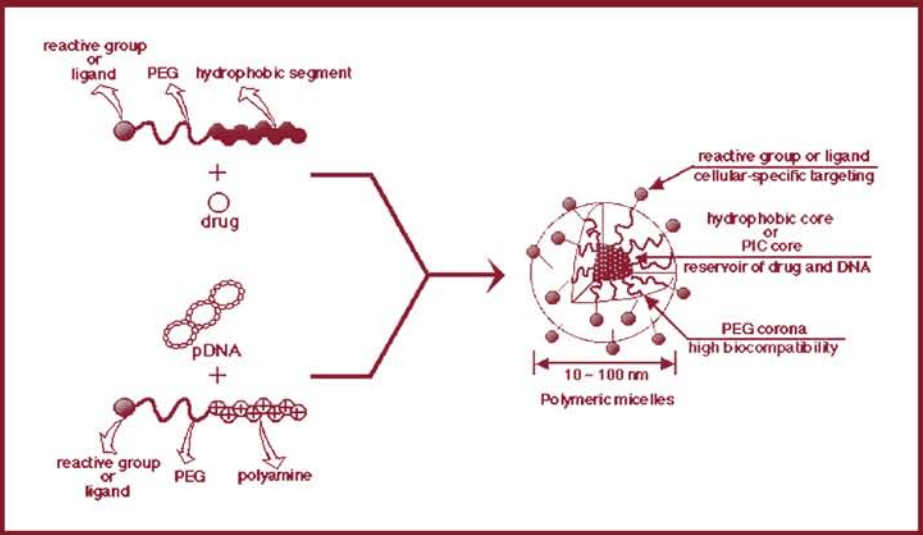
DRUGS AND THE PHARMACEUTICAL SCIENCES VOLUME 148

Polymeric Drug Delivery Systems



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Hydrogels for Oral Administration

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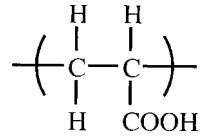
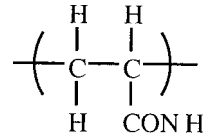
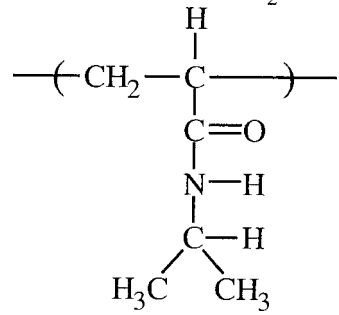
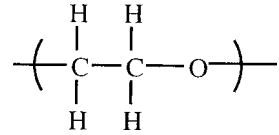
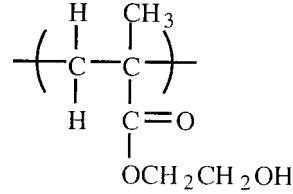
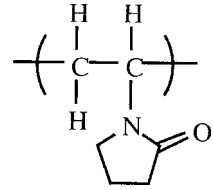
1. INTRODUCTION

1.1. Hydrogels

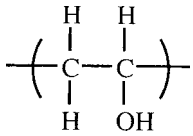
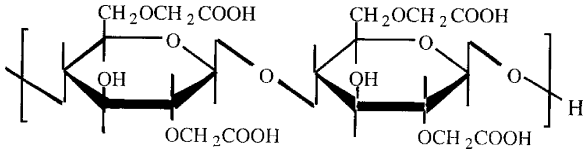
A hydrogel is a three-dimensional network of hydrophilic polymer chains held together by chemical bonds (i.e., covalent bonds) or physical bonding (e.g., hydrogen bonding, ionic interaction, and hydrophobic association) (1). Due to the hydrophilic nature of the polymer chains, the network is able to absorb water within its structure and swell without dissolving while maintaining the overall structure. Table 1 lists some examples of commonly used polymers that can be cross-linked to make hydrogels.

Table 1 Examples of Commonly Used Polymers for Making Hydrogels

Polymer name	Polymer structure
Poly(acrylic acid)	
Polyacrylamide	
Poly(*N*-isopropyl-acrylamide)	
Poly(ethylene oxide)	
Poly(hydroxyethyl methacrylate)	
Polyvinylpyrrolidone	

(*Continued*)

Table 1 (*Continued*)

Polymer name	Polymer structure
Poly(vinyl alcohol)	
Carboxymethyl-cellulose	

Hydrogels can be classified into different groups, just for convenience, based on the source of origin, chemical structure, preparation method, electric charge, physical structure, crosslinking, or function. There are two types of crosslinking: chemical and physical crosslinking. In the chemical crosslinking, all polymer chains are crosslinked to each other by covalent bonds, and thus, strictly speaking, each hydrogel is one molecule. For this reason, a hydrogel is sometimes called a "supermacromolecule." While chemical crosslinking is well defined by each chemical bond, physical crosslinking is made through multiple, simultaneous interactions of weaker bonding, such as hydrogen bonding and hydrophobic interactions. The area of such physical bonding among laterally associated polymer chains is known as a junction zone (2).

Hydrogels have been used widely in the development of drug delivery systems and biomedical devices. The water content in a hydrogel is at least 10% of the total weight. If the water content exceeds 95% of the total weight, the hydrogel is called superabsorbent. Due to the presence of water, the interfacial tension between the hydrogel surface and aqueous solution is very low, and this is one of the reasons why protein adsorption and cell adhesion to the hydrogel surface

is significantly reduced. The presence of water also minimizes the irritation to the surrounding tissue when a hydrogel is implanted into the body. The solid content in a swollen hydrogel ranges from about 90% to less than 1%, and thus the space, also known as an effective pore size, between polymer chains also varies. Hydrogels with an effective pore size in the ranges of 10–100 nm and 100 nm–10 μm are called microporous and macroporous hydrogels, respectively.

In the presence of abundant water, hydrogels absorb water and swell. This swelling process is the same as the dissolution of non-crosslinked hydrophilic polymers. Experimentally it is much easier to measure the weight of a swollen hydrogel than the volume, and thus the swelling ratio of hydrogels is usually expressed based on weights. The swelling ratio is defined as:

$$\text{Swelling ratio} = \frac{(\text{Weight of swollen gel})}{(\text{Weight of dried gel})}$$

One of the unique properties of hydrogels is that due to isotropic swelling, the original shape can be maintained during and after swelling.

1.2. Superporous Hydrogels

Hydrogels with the pore sizes larger than 10 μm are known as superporous hydrogels. The uniqueness of the superporous hydrogels is that the pores are interconnected to form an open cell structure, allowing extremely fast absorption of water into the center of the dried gel by capillary force. Figure 1 shows fast swelling property of a superporous hydrogel. The dried superporous hydrogel in Figure 1 swelled to 100 times of its dried weight (or volumes) within 30 s. This is something not possible with conventional dried hydrogels that do not have interconnected pores. Mechanical strength of a swollen gel can be improved by making superporous hydrogel composites (3,4) or superporous hydrogels with interpenetrating networks with the second polymer (5). The elastic property is useful in making mechanically strong superporous hydrogels more resilient to compression and elongation (6). The swollen

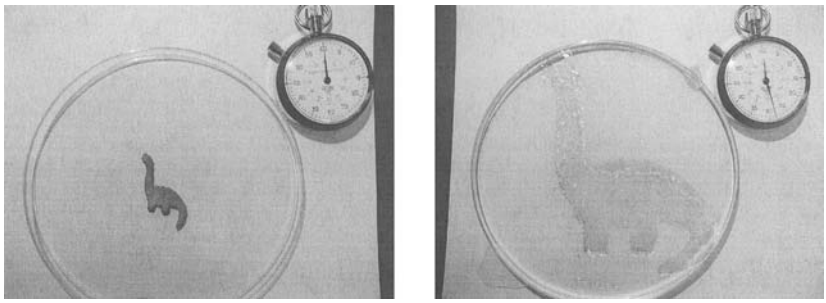


Figure 1 Swelling property of a dried hydrogel (left) to a larger size after swelling while maintaining the original shape (right) in aqueous solution.

hydrogel can be stretched to almost twice the original length without breaking.

2. APPLICATION OF HYDROGELS IN ORAL DRUG DELIVERY

Among all the routes of drug administration, oral drug delivery has been regarded as the most convenient method of drug administration. Drug delivery technologies are quite advanced enough to design dosage forms that can deliver drugs at a relatively constant rate for long periods of time ranging from days to months and even years. The time period of oral drug delivery, however, has been limited to a day. The maximum period of time available for drug absorption from an orally administered formulation is determined by the total transit time from mouth to colon, which is usually less than 24 h. Table 2 lists the residence time of both liquid and solid foods in each segment of the gastrointestinal (GI) tract (7). One of the properties of the GI tract is that the food contents remain in each part of the GI tract for different time periods. The values in Table 2 should be regarded as relative, not absolute, and are intended to emphasize general differences among different parts of the GI tract. Gastric emptying time ranges from 10 min to about 3 h depending on many factors,

Table 2 Transit Time in Each Segment of the GI Tract

Segment	Type of food	
	Liquid	Solid
Stomach	10–30 min	1–3 h
Duodenum	< 60 s	< 60 s
Jejunum and ileum	$3 \text{ h} \pm 1.5$ h	$4 \text{ h} \pm 1.5$ h
Colon		20–50 h

and when foods are present in the stomach, they have a tendency to delay the gastric emptying.

Development of once-a-day oral dosage forms is still a big challenge. Most drugs cannot be delivered for 24 h by a single administration, since oral dosage forms pass through the small intestine where most drug absorption occurs within a few hours. Once-a-day formulation is possible for some drugs, such as phenylpropanolamine and nifedifine, since they are absorbed well throughout the GI tract. Extending the residence time long enough for a drug to be absorbed from the upper small intestine remains an extensive research topic.

Recent advances in smart hydrogels have made it possible to exploit the changes in physiological uniqueness in different regions of the GI tract for the improved drug absorption as well as patient's convenience and compliance. Different hydrogels that are ideal in delivery of drugs to certain regions in the GI tract from oral cavity to colon are shown in Figure 2.

2.1. Oral Cavity

2.1.1. Fast-Melting Tablets

For more than a decade, fast-melting (also called fast-dissolving or fast-disintegrating) tablet technologies have been steadily advancing in the development of patient-friendly dosage forms. The fast-melting dosage forms, which can be administered easily without any water, are suitable for all age groups, but in particular for children, the elderly, and those who have difficulty in swallowing conventional tablets and capsules. The initial success of the first fast-melting

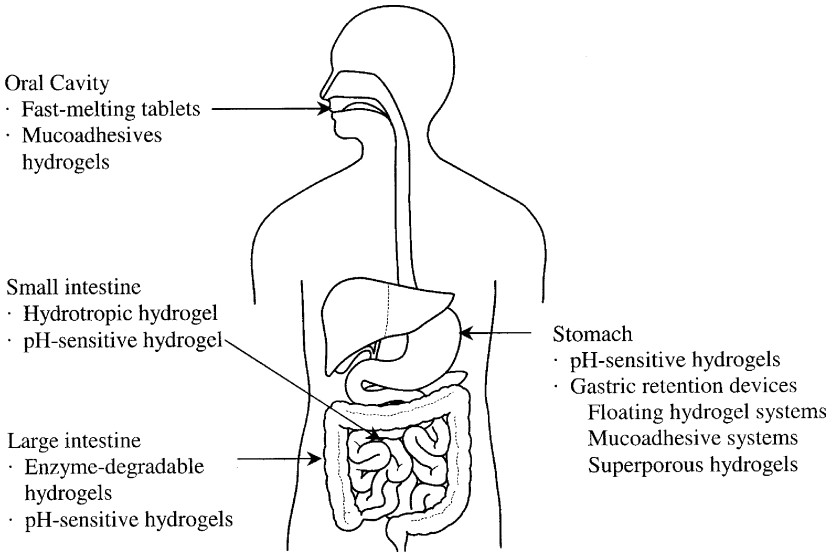


Figure 2 Different hydrogels and hydrogel formulations that can be used in different sites in the GI tract.

tablet formulation led to the development of different technologies. There are mainly three different technologies: freeze-drying, sublimation or heat molding, and direct compression. Freeze-drying technology produces tablets that can dissolve in a few seconds, while tablets produced by the sublimation and molding technology take a little bit longer to dissolve. The two technologies, however, are quite expensive and the prepared tablets are not mechanically strong. For this reason, direct compression technologies, which allow low cost of production and good mechanical roperties, are preferred, even though the melting time takes usually longer than 10 s, and sometimes more than 20 s.

To ensure the tablet's fast-melting property, water must be quickly absorbed into the tablet matrix. Current fast-melting tablet technologies are based on maximizing the porous structure of the tablet matrix and incorporating appropriate disintegrating agents and/or highly water-soluble excipients in the tablet formulation (8,9). Recently, microparticles of superporous hydrogels with fast swelling and super absorbent

properties were applied to develop fast-melting tablets by direct compression method (10). The size and shape of superporous hydrogel particles can be varied. Superporous hydrogels can be ground in dry state to make porous super disintegrant microparticles. The hydrogel struts in the superporous hydrogels have numerous pores smaller than 1 mm, which are connected to each other to maintain open pore structures. This unique porous structure allows for transport of water through capillary forces, resulting in an extremely fast wicking effect into the tablet core. Tablets prepared by direct compression in the presence of superporous hydrogel microparticles disintegrate in about 10–20 s due to the fast uptake of water into the core of the tablet (11). Since a lubricant has to be added for mass production, the effect of different lubricants on the disintegration time was examined. It was found that the presence of lubricants, especially hydrophobic lubricants, significantly delays the disintegration time. As shown in Figure 3,

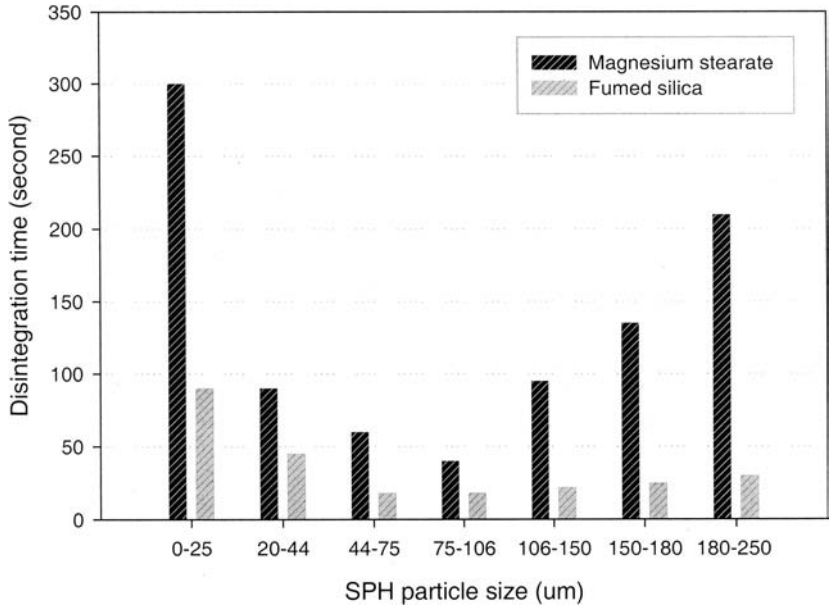


Figure 3 Effect of SPH particle size on the disintegration time of ketoprofen fast-disintegrating tablets.

the disintegration time is delayed to more than 100 s if magnesium stearate was used. On the other hand, fumed silica did not cause any such delays. In both cases, however, there were optimum particle size ranges for the fastest disintegration.

2.1.2. Buccal Drug Delivery Systems

In general the buccal tissue is regarded as more permeable to drugs than the skin. Buccal drug delivery may have faster onset of drug action and bypass the hepatic first-pass metabolism and degradation in the GI tract (12). One of the useful systems for increased patient compliance is bioadhesive dosage forms. Polymers that have been tested and used as mucoadhesives to the buccal tissue are hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC), polyvinylpyrrolidone, and poly(vinyl alcohol) (13), and mixtures of different polymers, such as HPC, hydroxypropylmethylcellulose (HPMC), karaya gum, and PEG 400 (14), or HPC and Carbopol 934 (15).

Mixing different polymers presents an advantage, since it can result in mucoadhesive hydrogel formulations even when each polymer alone does not form a hydrogel. For example, combining xanthan gum and locust bean gum resulted in formation of hydrogels, even though they do not form hydrogels individually (16). The formation of hydrogels was based on the double helix structure of xanthan gum and the straight chain of locus bean gum (14).

2.2. Stomach

The pH in the stomach, which is less than 3, is quite different from the neutral pH in the small or large intestine. This difference is large enough to be exploited in drug delivery using polyelectrolyte hydrogels. All the pH-sensitive hydrogels have pendant acidic (carboxylic or sulfonic acids) or basic (ammonium salts) groups that accept or release protons in response to changes in environmental pH. Poly(acrylic acid) becomes ionized as pH increases, while poly (*N*,*N*′-diethylaminoethyl methacrylate) (PDEAEM) becomes ionized as pH decreases (17).

Polycationic hydrogels swell less at neutral pH than at acidic pH; thus they minimize drug release at higher pH. This property was applied to mask poor tastes of some drugs in the neutral pH environment of the mouth. When caffeine was loaded into hydrogels made of copolymers of methyl methacrylate and *N*,*N*′-dimethylaminoethyl methacrylate (DMAEM), it was not released at neutral pH, but released with zero-order at pH 3–5 where DMAEM became ionized (18).

Polycationic hydrogels in the form of semi-interpenetrating polymer networks (semi-IPN) have also been used for drug delivery to the stomach. Semi-IPN of crosslinked chitosan and PEO showed more swelling under acidic conditions. This type of hydrogels could be applied for local delivery of antibiotics, such as amoxicillin and metronidazole, in the stomach for the treatment of *Helicobacter pylori* (19).

2.3. Gastric Retention Devices

Controlled-release drug delivery systems having prolonged gastric residence time have been studied extensively in both academia and industry (7). For many drugs that are mainly absorbed from the upper small intestine, such as drugs with absorption windows, controlled release in the stomach would result in improved bioavailability. Gastric emptying of oral dosage forms is known to be influenced most significantly by two main parameters: the physical properties (e.g., size and density) of the oral dosage form and the presence of food in the stomach (e.g., fasted or fed state). These parameters were exploited by various methods in the development of gastric retention devices.

2.3.1. Intragastric Floating Systems (Low-Density Systems)

In this system, the device is designed to float on top of the gastric juice due to its density being lower than that of water (7). One example of this type of devices is a single-unit hydrodynamically balanced system (HBS) which is composed of a drug, a hydrogel and other excipients (20). Commonly used

excipients are gel-forming or highly swellable cellulose-type hydrocolloids and polysaccharides.

2.3.2. Mucoadhesive Systems

A mucoadhesive system is an oral dosage form that is designed to stick to the mucosal surface of the stomach. If the dosage form can stick to the mucosal surface, then in the ideal case its gastric residence time would be increased until it is removed by turnover of mucins. The best mucoadhesive known so far is crosslinked poly(acrylic acid) (21), which is commercially available under polycarbophil (22) and Carbopol. They are highly mucoadhesive at pH 1–3 of the stomach. This is because poly(acrylic acid) interacts with mucins and other biomolecules through numerous simultaneous hydrogen bondings provided by carboxyl acid groups of poly(acrylic acid) at acidic pH (7). This non-specific mucoadhesive property turns out to be a deterrent in developing a useful mucoadhesive formulation, since the gel sticks to almost all surfaces it is in contact with. It is necessary to find polymers with a specific selectivity that they can adhere only to the mucus layer in the stomach.

2.3.3. Superporous Hydrogel Systems

Superporous hydrogels swell to a very large size with the swelling ratio being as high as a few hundreds or more. They also possess the fast swelling property that is required to avoid premature emptying by the housekeeper waves (3,4). This fast swelling property was applied to develop a gastric retention device that can remain in the stomach due to its large size after swelling.

Superporous hydrogels can be made to have high mechanical strength even after swelling. The mechanical strength of swollen superporous hydrogels can be improved by adding composite materials (4). One of the first useful composite materials was Ac-Di-Sol, which is crosslinked carboxymethylcellulose sodium with a hollow microparticulate shape. The hollow microparticles provide physical entanglements of polymer chains around the microparticles. This increased

the effective crosslinking density without making the superporous hydrogels too brittle. When tested in dogs, superporous hydrogel composites showed long-term gastric retention, ranging from several hours to a day (3,4). Biodegradable superporous hydrogels can be prepared by adding biodegradable crosslinkers, such as functionalized albumin (23).

2.4. Small Intestine

The small intestine, which is about 7 m long, is divided into the duodenum, the jejunum, and the ileum. The pH of the GI tract increases to an average of 6.9 in the duodenum, rising to 7.5 in the distal small intestine. The significant pH change from that in the stomach to around 7 has been exploited to develop the pH-dependent squeezing hydrogel system (24). pH-responsive hydrogel particles based on poly(acrylic acid) were used to develop pH-dependent silicone matrix (25). The release patterns of several model drugs having different aqueous solubilities and partitioning properties were correlated with the pH-dependent swelling pattern of the hydrogel particles. At pH 1.2, the network swelling was low and the release was limited to an initial burst. At pH 6.8, the network became ionized and higher swelling resulted in increased release. Sugar-based hydrogels, which are biodegradable copolymeric hydrogels based on sucrose acrylate, *N*-vinyl-2-pyrrolidinone, and acrylic acid, were investigated for oral drug delivery (26). A drug entrapped in the hydrogel showed a faster release profile in intestinal fluid than in the gastric fluid as a result of higher swelling in the intestinal fluid.

Hydrogels, especially superporous hydrogels, can also be used to develop peptide delivery systems through oral routes. Peptide drugs have been administered mostly by the parenteral route. Recently, peroral peptide delivery systems using superporous hydrogels were developed (27). Superporous hydrogels used in the systems increased their volume by about 200 times. Due to the volume increase, the gels were able to stick to the intestinal gut wall mechanically and deliver the incorporated drug directly to the gut wall. The

acid groups of poly(acrylic acid) of the superporous hydrogels is known to extract calcium ions from the gut wall to result in an opening of the tight junctions and also to deactivate the deleterious gut enzymes. After the peptide drugs have been delivered across the gut wall, the superporous hydrogels become over-hydrated and their structure become broken down by the peristaltic forces of the gut, and the remnants of the delivery systems are easily excreted together with the feces.

2.5. Large Intestine and Colon

The large intestine is divided into several parts: cecum, appendix, colon, and rectum. The major function of the colon is to absorb water and electrolytes. Primarily, absorption occurs in the proximal half of the colon (28). The surface area of colon is small, but due to the large residence time in the colon, drug absorption can be significant for some drugs. One of the important applications of drug delivery to the colon is the delivery of protein and peptide drugs including vaccines. A number of different methods, which utilize specific enzymes, different pH, and transit time of GI tract in different parts of the body individually or in combination, have been used for colon-specific drug delivery. Microbial enzymes present predominantly in the colon can be used for site-specific drug delivery. pH-sensitive polymers can protect drugs until they reach the colon. Based on the average transit time of 5 h from mouth to colon, a controlled release dosage form that can deliver a drug in the colon can be developed (29). Poly(acrylamide-co-maleic acid) and poly(*N*-vinyl-2-pyrrolidone-co-acrylamide-co-maleic acid) hydrogels showed a strong pH-dependent drug release behavior; i.e., minimum release at pH 2.0 but maximum release at pH 7.4 (30).

Colon-specific biodegradable hydrogels were prepared using azoaromatic crosslinkers (30). As shown in Figure 4, the hydrogels do not swell very well at low pH, so the release of drug will be minimized in the stomach. As the hydrogels move down the GI tract, they swell at higher pH due to ionization of the carboxylic acid groups. Since azoreductase

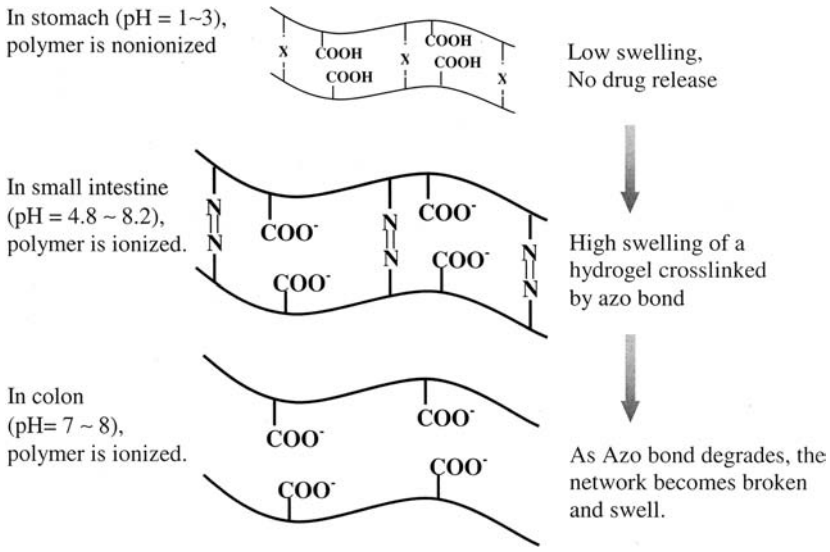


Figure 4 Schematic illustration of colon-specific drug delivery from biodegradable and pH-sensitive hydrogels. The azoaromatic moieties in the crosslinks are designated by –*N*=*N*–. (From Ref. 30.)

produced by microbial flora of the colon degrades azoaromatic crosslinkers of hydrogels, the hydrogels can be degraded only in the colon (31). The swelling kinetics of hydrogels can be controlled by the polymer composition and by the crosslinking density. Knowing the transit time, pH in the GI tract, diffusion rate of drugs through hydrogels, the rate of enzymatic degradation, and degree of swelling, one may be able to design drug delivery devices with desired release profiles at the desired time (32).

3. HYDROGELS FOR SPECIFIC APPLICATIONS

3.1. Hydrotropic Hydrogels for Delivery of Poorly Soluble Drugs

Drug molecules have to be dissolved in the GI tract to be absorbed. Poor aqueous solubility of hydrophobic drugs, which usually results in low bioavailability, has been one of

the limitations in designing clinically useful oral drug delivery formulations. Hydrotropes have been used to increase the aqueous solubility of poorly soluble drugs by 2–4 orders of magnitude (33,34). Application of low molecular weight hydrotropes in drug delivery, however, has not been practical, because it may result in absorption of a significant amount of hydrotropes themselves into the body along with the drug. One approach to prevent co-absorption of hydrotropes from the GI tract after oral administration is to make polymeric hydrotropic agents (hydrotropic polymers). Various polymeric solubilizing systems, such as polymeric micelles and micelle-like aggregates from polymeric amphiphilic agents, have been used to increase the solubility of poorly water-soluble drugs (35,36). Hydrotropic polymers and hydrogels are expected to provide an alternative method to increase the aqueous solubility of poorly soluble drugs for oral administration.

Hydrotropic hydrogels were examined to improve the aqueous solubility of paclitaxel (37). The loading of paclitaxel into the hydrogels was carried out by solubilizing paclitaxel in aqueous solutions of 2-(4-vinylbenzyloxy)-*N*-picolylnicotinamide) (2-VBOPNA) and 6-(4-vinylbenzyloxy)-*N*-picolylnicotinamide (6-VBOPNA), followed by the in situ crosslinking reaction to form hydrogels. As shown in Figure 5, paclitaxel solubility in hydrogels increased as the concentration of 2-VBOPNA or 6-VBOPNA used in hydrogel synthesis increased. The paclitaxel solubility was increased up to 1.62 mg/mL in the experiment, which is more than 5000 times that of paclitaxel solubility in water (37).

3.2. Alginate Hydrogels for Oral Vaccine Delivery

Peyer's patches, which are collections of lymphoid tissue containing B and T lymphocytes and macrophages, can be found in duodenum, jejunum, and ileum. These areas may be targets for protein and peptide drug delivery including oral vaccines (38). Lymphoid tissues analogous to Peyer's patches can be found in the large intestine as well. Specialized epithelial cells in the dome region of the patch, called follicle associated

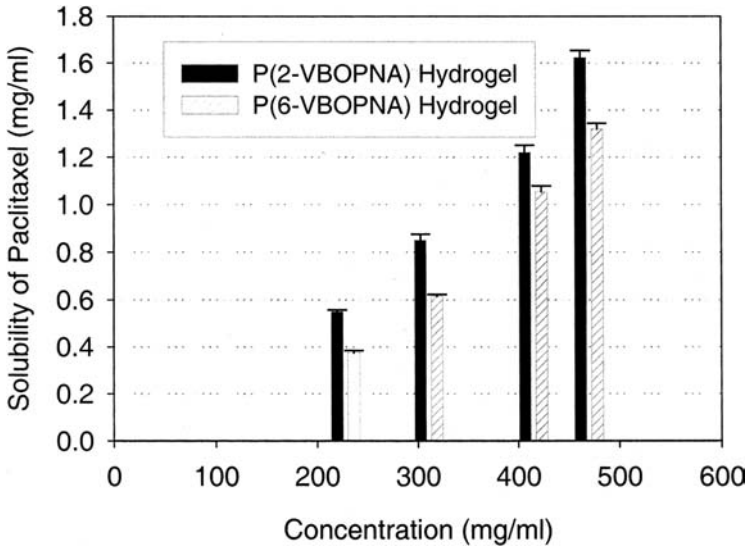


Figure 5 Paclitaxel solubility in *N*-picolylnicotinamide hydrogels as a function of the concentrations of 2-VBOPNA and 6-VBOPNA. (From Ref. 37.)

epithelial or microfold cells, pick up antigens and transport them to the underlying lymphoid tissue (38).

Immunization has been relied on in the induction of humoral immunity by parenteral administration of vaccines. Antibodies from this do not necessarily reach the mucosal surfaces of the GI tract where most infectious agents encounter the host first. Secretory IgA (sIgA) is the dominant antibody isotype present at mucosal sites. Since sIgA inhibits the attachment of bacteria and viruses to mucosa and neutralizes toxins, mucosal immunity can provide the first line of immunological defense. However, induction of immunity at mucosal surfaces needs administration of vaccines directly to the mucosal site.

Since vaccines are quite susceptible to low pH in the stomach and enzymatic degradation in the GI tract, various methods have been tried to improve the vaccine delivery. Alginate microspheres have been examined to deliver vaccines by oral administration (38). Vaccine-containing

alginate hydrogel microspheres were shown to be effective for oral vaccination in several animal species. This method has several advantages as compared with other methods. The alginate vaccine delivery system removes the use of organic solvents or high temperatures, which are often needed for the preparation of microparticles using other methods. Since an aqueous environment is used throughout the preparation process, this method can also be used with live bacteria and viruses. The alginate microspheres are able to protect vaccines from degradation in the GI tract. Since microparticles with a diameter 1–10 μm are known to be absorbed well by the Peyer's patches, special attention has been paid to making small-sized particles (39).

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5

Hydrogels for the Controlled Release of Proteins

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1. INTRODUCTION

Modern biotechnology has resulted in the production of a great variety of pharmaceutically active proteins (1). Recent statistics show that the Food and Drug Administration (FDA) has approved 130 biotechnology-derived protein medicines and vaccines (2). The unfavorable biopharmaceutical properties of these protein drugs, however, have severely hampered their therapeutic and clinical application. First of

all, oral administration is hardly possible due to chemical and enzymatic degradation in the gastrointestinal tract. Second, proteins have a short half-life after parenteral administration (e.g., intravenous injection), which makes repeated injections or continuous infusion of the protein necessary to obtain a therapeutic effect (3). To overcome these problems, a large number of delivery systems have been designed and evaluated for the release of proteins (4–6). A frequently investigated polymer involved in the design of controlled-release systems for proteins is poly(lactic-co-glycolic acid), or PLGA. This polymer, however, has some intrinsic drawbacks as a protein-releasing matrix. Organic solvents have to be used to prepare pharmaceutical dosage forms (e.g., microspheres), and a low pH might be generated inside the matrix during degradation (7,8). Both factors adversely affect the structural integrity of the protein to be delivered. Moreover, it is difficult to manipulate the release of a protein from PLGA matrices. As an alternative for these biodegradable polyesters, hydrogels (crosslinked, hydrophilic polymeric networks) have been proposed as protein-releasing matrices. This chapter summarizes the work carried out on biodegradable hydrogels based on dextran (dex) and amphiphilic poly(ether ester) multiblock copolymers for protein delivery.

2. HYDROGELS: GENERAL FEATURES

Hydrogels are polymeric networks that absorb and retain large amounts of water. In the polymeric network, hydrophilic groups or domains are present that are hydrated in an aqueous environment, thereby creating the hydrogel structure. As the term “network” implies, crosslinks have to be present to avoid dissolution of the hydrophilic polymer chains/segments into the aqueous phase. Because of their water-absorbing capacity, hydrogels have been found to have widespread applications in different technological areas; hydrogels have been used as materials for contact lenses and protein separation, as matrices for cell encapsulation, and as devices for the controlled release of drugs and proteins (9–14). As

mentioned, crosslinks have to be present in a hydrogel in order to prevent dissolution of the hydrophilic polymer chains in an aqueous environment. A great variety of establishment of crosslinking has indeed been used to prepare hydrogels. Crosslinking can be established either by chemical means (e.g., by introducing chemical linkages between different polymer chains) or by physical interactions (15). When the protein-loaded gels are administered by injection, the advantages that correspond to hydrogels' biodegradability become obvious. Therefore, labile bonds are frequently introduced in the gels. The labile bonds can be broken, in most of the cases by chemical or enzymatic hydrolysis, under physiological conditions. But degradability as such is not the ultimate solution. Once the hydrogels are implanted, it is of the utmost importance that the gels have good biocompatibility and that the formed degradation products have a low toxicity. In general, hydrogels possess good biocompatibility because their hydrophilic surface has a low interfacial free energy in contact with body fluids, a characteristic that results in a low tendency for proteins and cells to adhere to these surfaces. Moreover, the soft and rubbery nature of hydrogels minimizes irritation to surrounding tissue (16,17). The nature of the formed degradation products can be tailored by a rational and proper selection of the hydrogel building blocks.

3. DEXTRAN HYDROGELS AS PROTEIN-RELEASING MATRICES

3.1. Introduction

During the past decades, many studies have been devoted to the release of proteins from degradable hydrogels based on dextran or dex. An important reason to use dex as building blocks for hydrogels concerns the low or absent toxicity; not surprisingly, then, dex has been used as a plasma expander (18). Initially, research was focused on the use of enzymatically degrading hydrogels that were crosslinked by gamma irradiation or by the free radical polymerization of (meth)acrylate functionalized dex and degraded by the action of the

enzyme dextranase (19–23). An enzymatically degrading bovine serum albumin (BSA)-loaded hydrogel that was crosslinked by a condensation reaction between activated dex and 1,10-diaminodecane has also been reported (24). The required enzyme dextranase is of bacterial or fungal origin and present in the human colon (25). Therefore, dex-based gels are under investigation as a colon delivery system (24,26). For delivery at other sites, dextranase has to be co-encapsulated in the hydrogel system in order to control the release of the entrapped protein. However, dextranase is only active in hydrogels with a relatively low crosslink density (21,27,28). Moreover, the use of dextranase might evoke unwanted immune responses to this protein. In order to circumvent the use of dextranase in parenteral systems, current research is now focused on the non-enzymatic degradation of dex hydrogels, i.e., on hydrogels that degrade and release their contents upon hydrolytic cleavage of the crosslinks. In the following sections, we will differentiate between chemically and physically crosslinked hydrogels that are hydrolytically sensitive and discuss their use as protein-releasing matrices.

3.2. Chemically Crosslinked Dextran Hydrogels

Methacrylate groups coupled to water-soluble polymers such as dex (structure given in Fig. 1) are sensitive toward hydrolysis under physiological conditions (29). However, after polymerization, the methacrylate esters are very resistant to hydrolysis (30). This means that gels derived from these polymers can only degrade under physiological conditions once the polymer main chains are hydrolyzed by a matching enzyme (dextranase). In order to increase sensitivity toward hydrolysis, we introduced degradable units between the methacrylate groups and the dex backbone, i.e., a carbonate ester in dex derivatized with hydroxyethylmethacrylate (HEMA) (dex-HEMA) or a combination of a carbonate ester and lactic acid groups in dex derivatized with HEMA-lactate (dex-lactate-HEMA) (Fig. 1) (31). After polymerization of these derivatives, the gels degraded under physiological

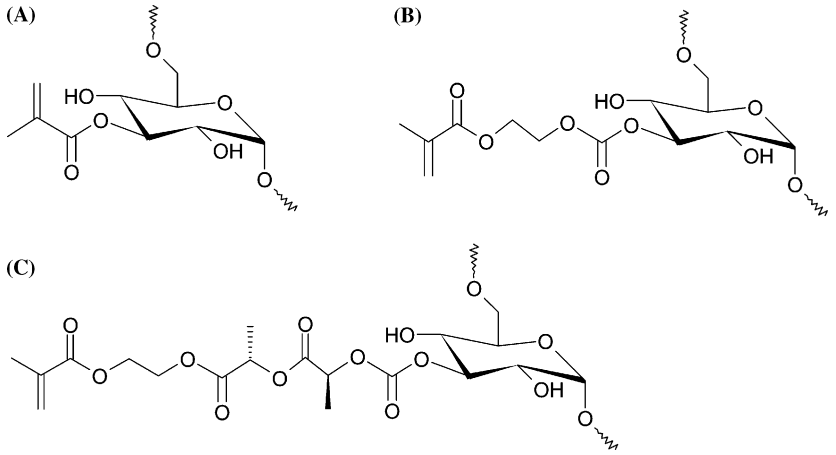

Figure 1 Structure of (A) dexMA, (B) dexHEMA and (C) dex(lactate)$_2$HEMA.

conditions, owing to the presence of (carbonate) ester groups in the crosslinks, and yielded dex, lactic acid, and short fragments of polyhydroxyethylmethacrylate (pHEMA) as degradation products (schematically shown in Fig. 2).

The degradation time varied from 1 day to more than 3 months and could be controlled by the type of ester group in the crosslinks, the crosslink density of the gel, and the length of the lactic acid spacer (30,32). Interestingly, the gels had good biocompatibility both in the form of implants and in the form of injectable microspheres, and the degradation time in vivo (after subcutaneous implantation in rats) was about the same as the degradation time in vitro (33,34). This means that, also in vivo, the degradation is most likely caused by chemical hydrolysis.

The release of a model protein [immunoglobulin G (IgG)] from degrading, cylinder-shaped (radius 0.23 cm, length 1 cm) dex-lactate-HEMA hydrogels with varying initial water content and degrees of substitution (DS, average number of HEMA-lactate groups per 100 glycopyranose residues) was investigated (30,35). Representative release profiles are shown in Figure 3. From this figure, it appears that, for gels with a high initial water content, a first-order release of the

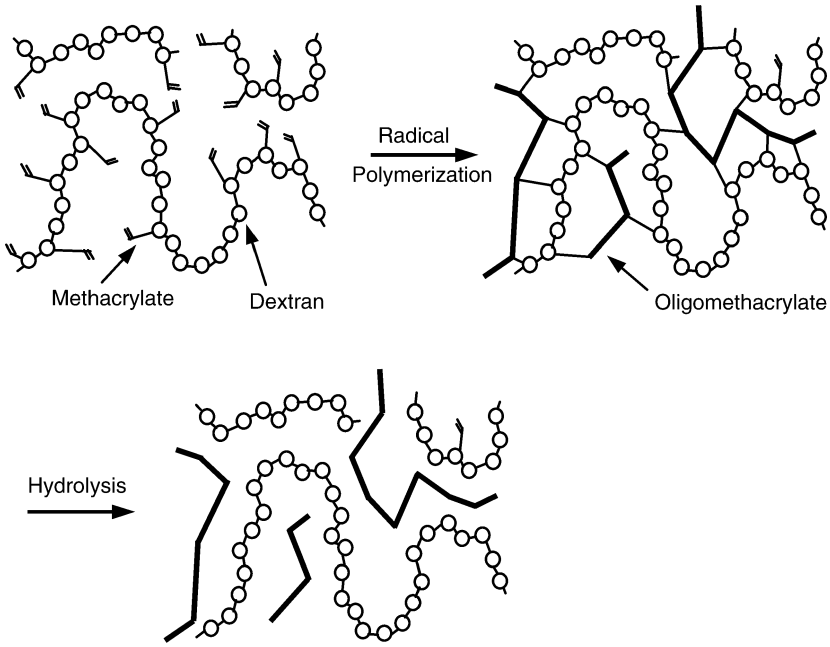

Figure 2 Schematic representation of the formation and degradation for dex(lactate)HEMA hydrogels. Gels are formed by radical polymerization of the methacrylate groups using KPS and TEMED as initiator system. Degradation occurs by hydrolysis of the carbonate and lactate ester.

protein is to be observed (diffusion-controlled release), whereas for gels with a lower initial water content, an almost zero-order release is to be observed for 35 days (degradation-controlled release).

Protein-loaded injectable microspheres can be prepared in an all-aqueous system as schematically shown in Figure 4 (36). Because the protein is present during the hydrogel formation, it can be entrapped in pores of the gel that are smaller than the protein.

Figure 5 shows the release of IgG from different degrading dex-HEMA microspheres at pH 7.0 and 37°C. Interestingly, dex-HEMA microspheres showed a delayed release of the entrapped protein. The delay time increased with an increasing degree of methacrylate substitution, a correspon-

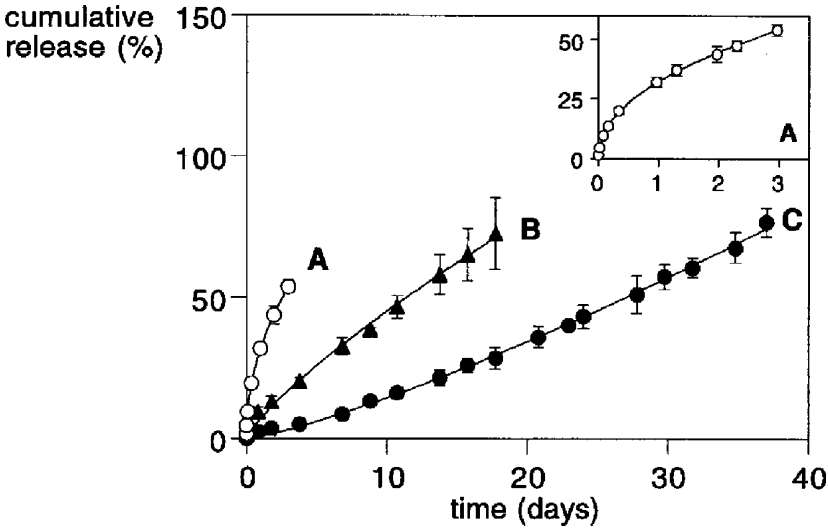

Figure 3 Cumulative release of IgG in time from dex-lactate-HEMA hydrogels (DS 10) with initial water content 90% (A), 80% (B), and 70 % (C). (From Ref.30.).

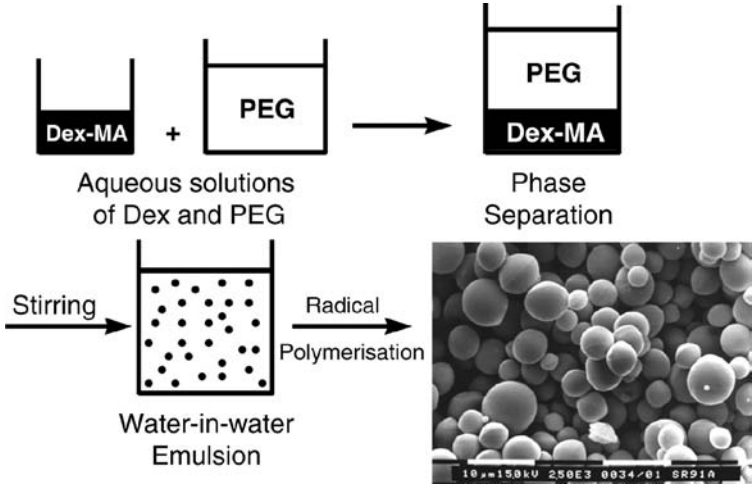

Figure 4 Schematic representation of the microsphere preparation process.

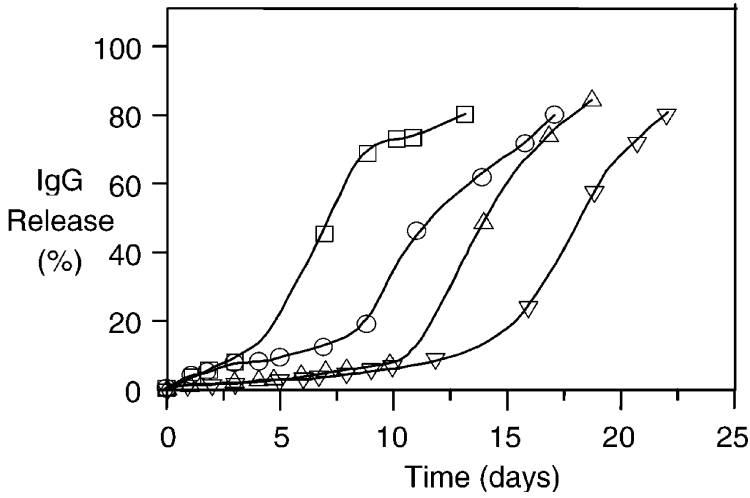

Figure 5 Cumulative release of IgG in time from degrading dex-HEMA microspheres [water content 50% (w/w)] in time DS 3 (□), DS 6 (○), DS 8 (△), and DS 11 (▽). (From Ref. 35.)

dence that demonstrates that the release of the protein was fully controlled by the degradation rate of the microspheres (35).

In vivo experiments using DBA/2 mice with Sutton lymphoma 2 (SL2), lymphosarcoma, revealed that the cytokine interleukin-2 (IL-2), which was slowly released from dex-HEMA microspheres over a period of 3–5 days, had comparable therapeutic effects as repeated injections of the free protein were administered for 5 consecutive days (37). This finding demonstrates that the biological activity of the released protein was preserved.

Another route to dex-based hydrogels was published by Zhang and colleagues. They modified dex with a polymerizable group, either acryloyl chloride or allyl isocyanate (38,39). The dex derivatives were dissolved in dimethyl formamide (DMF) together with a poly(D,L-lactic acid)diacrylate macromer (PDLLAM). A network was obtained by ultraviolet (UV)-induced polymerization. The swelling of the gels depended on, among other things, the ratio of dex/PDLLA

in the network, the degree of the substitution of dex with the polymerizable group, and the UV irradiation time (38). These gels were investigated as a matrix for the release of albumin and insulin (40,41). The release was dependent on the gel composition and was governed by a combination of diffusion and the degradation of the matrix. Although Zhang and colleagues claim that these hydrogels are biodegradable, complete biodegradability was not observed and is unlikely owing to the choice of dex derivatives.

The chemically crosslinked dex hydrogels discussed here were prepared by the free radical polymerization of the methacrylate units under mild conditions (room temperature, pH 7), using either UV irradiation in the presence of a photoinitiator or an initiator system composed of a peroxydisulfate and *N*,*N*,*N*′,*N*′-tetramethylenediamine (TEMED). However, unreacted peroxydisulfate and TEMED as well as their degradation products, have to be extracted from the gel before in vivo application. Moreover, these initiator systems can also damage proteins once they are present during preparation of the gels, as will be further discussed in Sec. 5. Therefore, we recently developed physically crosslinked dex hydrogels that avoid the use of harmful crosslinking agents, as will be described in the next section.

3.3. Physically Crosslinked Dextran Hydrogels

In physically crosslinked gels, dissolution of the polymer network is prevented by physical interactions that exist between the polymer chains. Physical crosslinking can be established by, for instance, ionic, hydrophobic, or coiled-coil interactions (15). A novel physical method by which to create hydrogel is the use of stereocomplex formation. This method has been recently investigated by us and others (42–46). In this section, the results obtained with these gels based on dex are summarized.

The novel hydrogel system, under investigation within our department, is schematically shown in Fig. 6.

Importantly, the hydrogel is formed in an all-aqueous environment in which the use of organic solvents is avoided. Crosslinking is established by stereocomplex formation

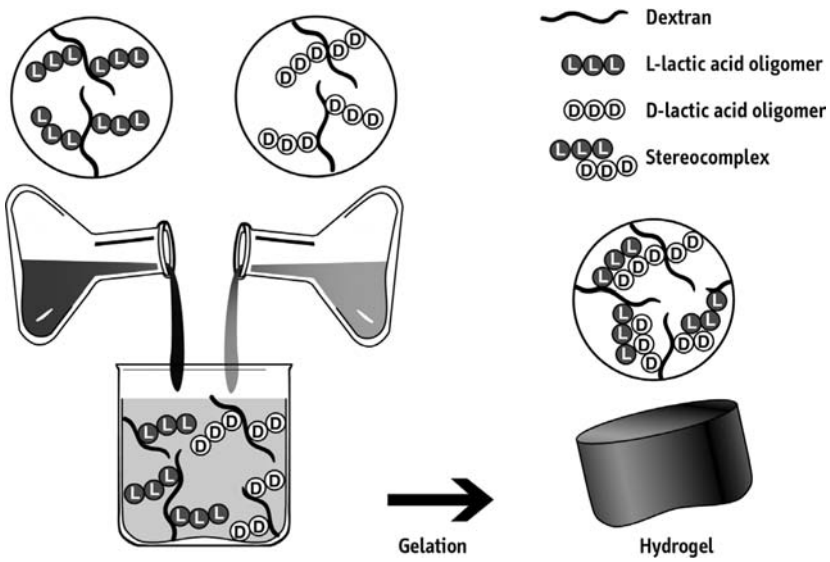

Figure 6 The concept of the stereocomplex hydrogel system. The hydrogel is simply obtained after mixing aqueous solutions of dex-(L)-lactate (L-lactic acid oligomer grafted to dextran) and dex-(D)-lactate.

between lactic acid oligomers of opposite chirality grafted to dex (42). Figure 7 shows the chemical structure of the synthesized dex-lactate products characterized by their degree of the polymerization (DP) of the lactate graft and by their DS. The hydrogel system is expected to be fully biodegradable, since the lactic acid oligomers (that will be degraded to lactic acid) are coupled to dex via a hydrolytically sensitive carbonate ester bond. These features make this hydrogel system very suitable for the controlled release of pharmaceutically active proteins.

Protein-loaded stereocomplex hydrogels were simply prepared by dissolving the protein in the dex-lactate solutions prior to mixing. It was shown that, under physiological conditions, the gels are fully degradable (46). The degradation time depended on both the pH and the composition of the hydrogel (i.e., on the number of lactate grafts, the length and polydispersity of the grafts, and the initial water content) and varied from 1 to 7 days (Fig. 8). Under non-degrading conditions

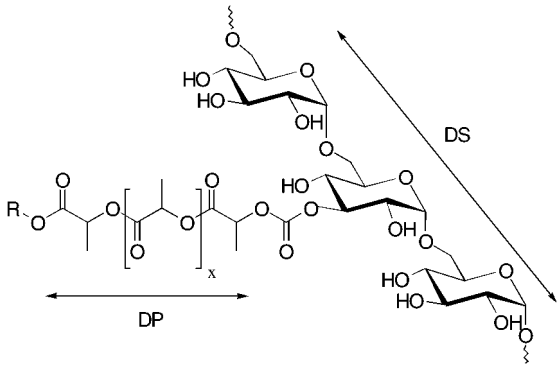

Figure 7 Dex-lactate product with DP = x + 2 and DS (degree of substitution; the number of lactate grafts per 100 glucopyranose units).

(pH 4), the hydrogels, having a water content of almost 90% in their swollen states, were stable for more than 1 month.

As shown in Figure 9, the gels showed a release of the entrapped model proteins (IgG and lysozyme) over 6 days,

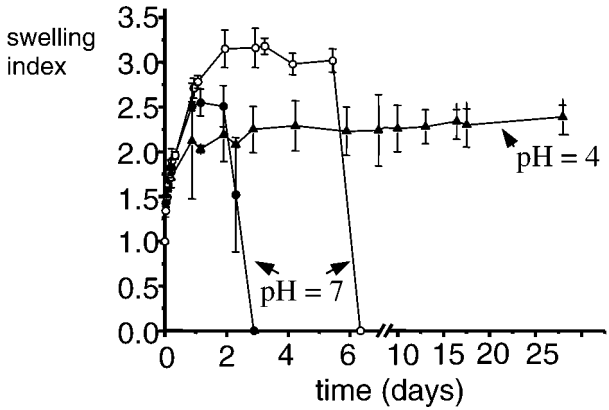

Figure 8 Swelling behaviour of dex-lactate stereocomplex hydrogels (DS = 6, 70% water 37°C): high polydispersity lactic acid grafts ($DP_{average} = 12$, $M_w/M_n \approx 1.25$, filled symbols) and low polydispersity grafts (DP = 11 to 14, $M_w/M_n \approx 1.01$, open circle). The filled triangles represent swelling under non-degrading conditions (pH 4). (From Ref. 46.)

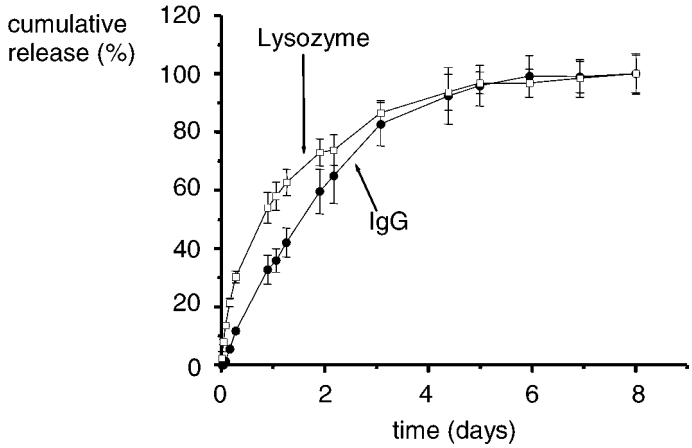

Figure 9 Release profiles of lysozyme (open squares) and IgG (filled circles) from dex-lactate stereocmplex hydrogel with low polydispersity grafts (DS = 6, DP = 11 to 14, 70% water pH 7, 37°C). (From Ref. 46.)

and the kinetics depended on the gel characteristics, such as the polydispersity of the lactate grafts and the initial water content. The release of lysozyme was by diffusion, whereas for the bigger IgG, whose hydrodynamic radius approaches the estimated mesh size of the hydrogels, swelling/degrada-degradation as well, played a role in the release. Importantly, the proteins were quantitatively released from the gels and with full preservation of the enzymatic activity of lysozyme, a fact that emphasizes the protein-friendly preparation method of the protein-loaded stereocomplex hydrogel.

In a recent study, we investigated the therapeutic efficacy of recombinant human IL-2 (rhIL-2)-loaded, in situ gelling, physically crosslinked dex hydrogels, locally applied to SL2 in mice (47). As a control, free rhIL-2 was administered locally in either a single injection or on 5 consecutive days. All mice received the same total dose of rhIL2. The rhIL2-loaded hydrogels released most IL-2 over a period of about 5 days. The biocompatibility and the biodegradability of the gels were excellent, as there was no acute or chronic inflammatory reaction and as the gels were completely replaced by fibroblasts after 15 days. The therapeutic efficacy of

rhIL2-loaded in situ gelled hydrogels is very good, as was demonstrated in DBA/2 mice bearing SL2. The therapeutic effect of a single application of gels loaded with 1×10^6 IU rhIL2 is at least comparable to the therapeutic effect of the injection of an equal dose of free rhIL2 (47).

4. AMPHIPHILIC POLY(ETHER ESTER) MULTIBLOCK COPOLYMERS AS PROTEIN-RELEASING MATRICES

4.1. Introduction

Physically crosslinked hydrogels can be obtained from multiblock copolymers or graft copolymers. Among them, multiblock copolymers consisting of repeating blocks of hydrophilic poly(ethylene glycol) (PEG) and hydrophobic poly(butylene terephthalate) (PBT) have been studied extensively for controlled-release applications (48–58). In vitro and in vivo studies have shown that PEG/PBT copolymers are well tolerated and do not cause adverse tissue or systemic effects (59–62). The poly(ether ester)s are biodegradable and degrade by hydrolysis (ester bonds) and oxidation (ether bonds) (63). The copolymers, also known under the registered trademark PolyActive^{TM}, have been used in a wide range of biomedical applications, including bone (64), cartilage (65), and skin repair (62). Medical devices made of PEG/PBT copolymers have been approved by both the FDA in the United States and Notified Bodies in Europe.

The poly(ether ester) multiblock copolymers are synthesized by the polycondensation of dimethyl terephthalate (DMT) with PEG and 1,4-butanediol (BD). An important advantage of multiblock copolymers is the possibility of varying the amount and the length of each of the building blocks, enabling the manufacturing of a wide range of customized polymers. In the case of PEG/PBT copolymers, this can be obtained by varying the feed ratio of PEG to BD or by adjusting the molecular weight of the hydrophilic PEG blocks. The copolymers are abbreviated as **a**PEGT**b**PBT**c**, in which **a** is the PEG molecular weight, **b** the weight % poly(ethylene glycol)-terephthalate (PEGT), and **c** the weight % PBT (Fig. 10).

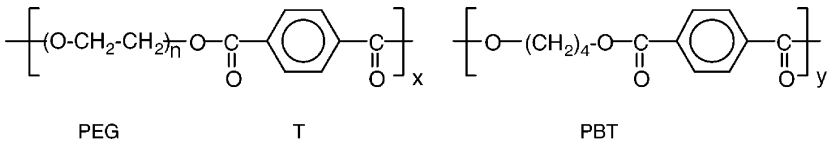

Figure 10 Chemical structure of the block that from PEG/PBT multiblock copolymers n determines the molecular weight of the PEG segment, x and y determine the weight ratio between PEGT and PBT.

Characteristics such as rate of degradation, swelling, and mechanical strength can be precisely controlled by a proper combination of the two copolymer segments. Thus, a tight control over the release rates of diverse molecules, including vitamins, peptides, and proteins, can be obtained (50,54,56,58).

4.2. Protein Release from Poly(Ether Ester) Hydrogel Films

Protein-containing PEG/PBT matrices (films or microspheres) were prepared according to an emulsion method (50). Polymer solutions in chloroform or dichloromethane were mixed with aqueous protein solutions to create a water-in-oil (w/o) emulsion. The emulsions were either cast onto a glass plate to form a film or dispersed in an aqueous solution to produce microspheres according to a water-in-oil-in-water (w/o/w) emulsion method. As demonstrated for a model protein (lysozyme) that was entrapped in films (approximately 100 μm in thickness), the release rate increased with both the increasing PEGT content of the copolymer and an increase in the molecular weight of the PEG segments (Fig. 11). This was explained by the difference in the degree of the copolymers' swelling in water (50). Swelling, and consequently the permeation of solutes through the hydrogel, increased as the molecular weight of the PEG segments or the PEG content increased (51).

For highly swollen copolymers, the release of lysozyme was complete within 1 day and followed first-order kinetics. Polymers that were swollen to a lesser extent released

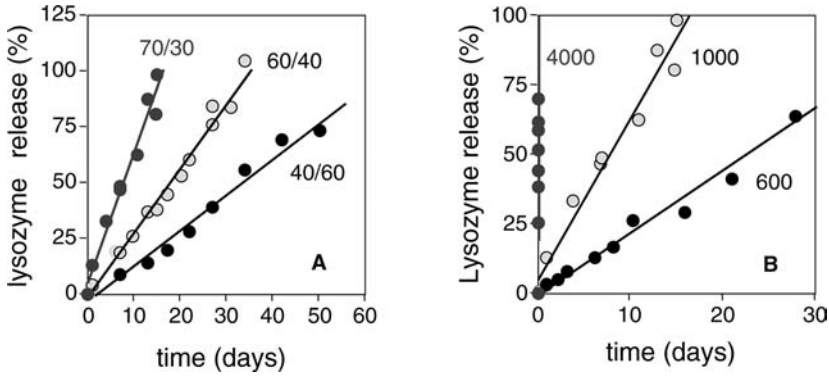

Figure 11 Cumulative release of lysozyme from PEG/PBT films in PBS (pH 7.4) at 37°C (A) M_w of the PEG segment is 1000 g/mole and PEGT/PBT ratio is 70/30; 60/40 or 40/60; (B) PEGT wt% is fixed between 70 and 80% and M_w of the PEG segment is 600, 1000 or 4000 g/mole. (Adapted from Ref. 50.)

lysozyme with an almost constant rate (near zero-order release). This release behavior was attributed to a combination of protein diffusion and polymer degradation (50). However, the mass loss observed during the in vitro release periods was very limited (51). Therefore, the effect of polymer chain scission on protein diffusion was studied. Polymers of different molecular weights were obtained by incubating 1000PEGT70PBT30 in phosphate buffered saline (PBS) solutions at 37°C for various time periods. Subsequently, the predegraded polymers were used to prepare protein-containing films. The lysozyme release rate of films increased with the decreasing molecular weight of the polymers. Figure 12 shows the diffusion coefficient of lysozyme (D), calculated from the release curves, as a function of the polymer molecular weight (M_n).

A linear relationship was observed between D and M_n^2. Taking into account the polymer degradation during release, M_n can be written as a function of time, resulting in a time-dependent diffusion coefficient. The new equation for the time-dependent diffusion coefficient was incorporated into solutions of the diffusion equation that could adequately describe lysozyme release from poly(ether ester) copolymers (50).

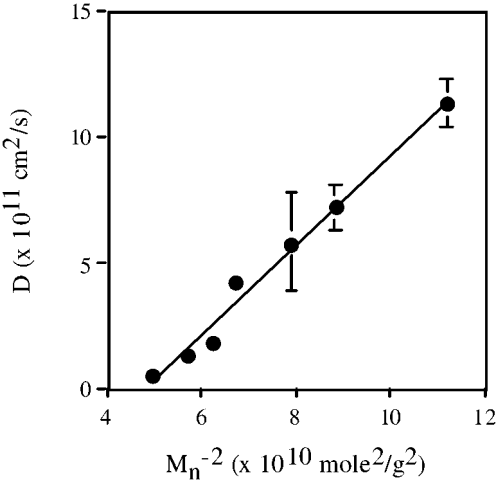

Figure 12 Effect of polymer molecular weght on lysozyme diffusion coefficient (1000PEGT70PBT30). (From Ref. 50.)

The release mechanism was explained by assuming that chain scission decreased the amount of effective crosslinks or entanglements present in the swollen copolymer matrix. Therefore, the resistance to the diffusion of proteins through the copolymer hydrogels was reduced. In the case of the observed zero-order release, the increasing permeability of the matrix in time may have compensated for the decline in the release rate caused by the reduced protein concentration in the matrix, to yield a constant release rate (50).

4.3. Protein Release from Poly(Ether Ester) Microspheres

Protein-containing PEG/PBT microspheres were prepared according to the well-known double-emulsion method (52,53). In contrast to what is often observed for hydrophobic polymers like PLGA, dense poly(ether ester) microspheres were obtained. This outcome was explained by the amphiphilic character of the polymers, which created very stable emulsions. The latter is considered a prerequisite for the formation of non-porous microspheres. A drawback of applying the w/o/w emulsion technique for the production of protein-

loaded microspheres is related to the entrapment efficiency. In the case of polymers with a relatively low degree of swelling (e.g., less than 100% swelling), entrapment efficiencies close to 100% were observed for lysozyme. However, for highly swollen microspheres, only 10% of the lysozyme was effectively entrapped within the microspheres. This finding was ascribed to a premature release of lysozyme during the 2 h, during which the solvent in PBS evaporated. The release during preparation increased with the increasing equilibrium water content of the matrix owing to a greater diffusivity. In order to encapsulate small hydrophilic drugs in highly swollen PEG/PBT microspheres, other methods should be applied—methods that avoid in-water hardening of the spheres.

In general, microspheres released proteins and peptides in a similar fashion as films (50,52–55). A continuous release could be obtained, without an initial burst, for periods ranging from days to months. Besides, by changing the copolymer composition or the molecular weight, the release profile could be altered by a variation of the w/o emulsion composition (56). An increase in the water content of the emulsion (the water/polymer ratio, w/p) increased the water uptake of the microspheres. At low w/p, this had no effect on the release rate. However, when the w/p was above a critical value of 1–1.5 mL/g, the release kinetics was altered. Figure 13 shows that the rate of the BSA release from microspheres increased with increasing w/p. Interestingly, a delayed release was observed for the formulation with the lowest w/p. Such a delayed release may be used to develop vaccine formulations with intrinsic booster effects.

4.4. In Vivo Protein Release

In order to obtain an in vivo/in vitro correlation for PEG/PBT multiblock copolymers, radiolabeled lysozyme was incorporated into microspheres (57). Three different compositions were selected to produce microspheres with distinct release rates. The release of lysozyme in vitro (PBS, pH 7.4, 37°C) was essentially complete within 1 week for a 1000PEGT70PBT30 composition, whereas release continued for 3–4 weeks for both the

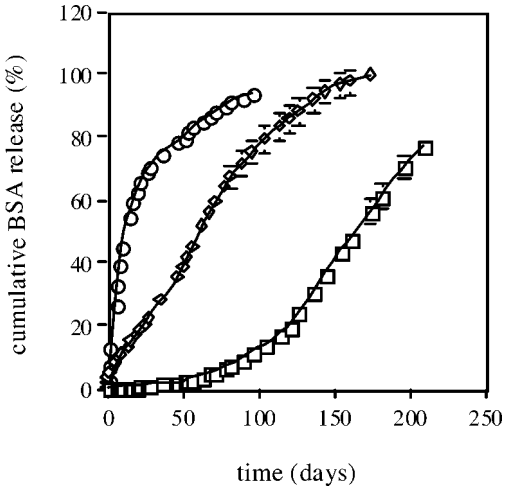

Figure 13 Effect of emulsion w/p on the release of BSA from 1000PEGT70PBT30 microspheres in PBS at 37°C. Matrices were prepared from emulsions with a w/p of 1.75 ml/g (○), 1.5 ml/g (△) and 1.25 ml/g (□). (From Ref. 56.)

1000PEGT60PBT40 and the 600PEGT77PBT23 microspheres. After rats were given subcutaneous injections, ^{14}C methylated lysozyme could be detected beyond 14 days after the dose for the 1000PEGT70PBT30 microspheres but were measurable up to the last sample point (28 days) for the 1000PEGT60PBT40 and the 600PEGT77PBT23 microspheres (Fig. 14).

On the basis of plasma radioactivity concentrations after injection of lysozyme-containing microspheres and the observed clearance rate of free ^{14}C lysozyme, the cumulative in vivo release was determined. A comparison of the release profiles showed an excellent congruence between release in vitro in PBS and in vivo in rats (Fig. 15). Very often for PLGA microspheres, in vivo release differs from in vitro release insofar as shape and/or time-course are concerned. In contrast to PLGA-based microspheres, protein diffusion through the swollen PEG/PBT matrix is the main rate-controlling factor, and this conclusion may explain the good correlation. However, the results for the release of lysozyme from PEG/PBT microspheres cannot be directly extrapolated to other

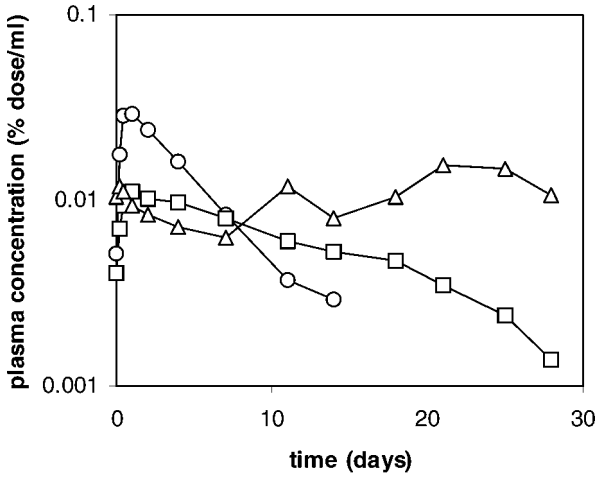

Figure 14 Mean plasma radioactivity concentration in male rats after a single subcutaneous dose of ^{14}C-methylated lysozyme, entrapped in 1000PEGT70PBT30 (○) 1000PEGT60PBT40 (△) 600PEGT77PBT23 (□) microspheres. (From Ref. 57.)

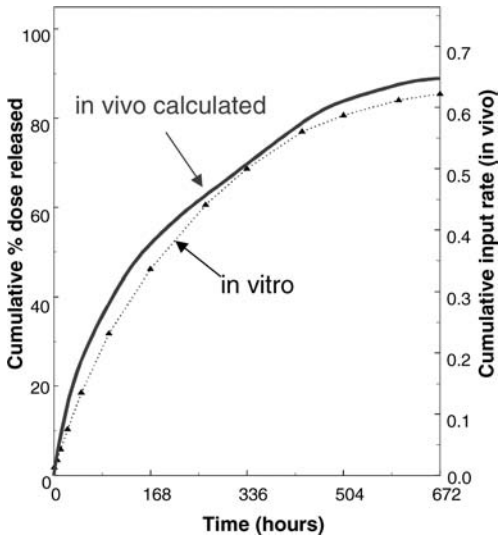

Figure 15 Comparision of in vitro cumulative release and in vitro cumulative input of radioactivity for 1000PEGT60PBT40 microspheres. (From Ref. 56.)

proteins. In vivo, the protein-dependent metabolism and immune response will affect the protein plasma levels. Furthermore, the model protein (lysozyme) that was studied was fairly small. Ultimately, the size of the protein may determine the route of transport through the tissue into the blood stream.

4.5. Tissue Engineering

At present, various groups study extensively the application of PEG/PBT copolymers in tissue engineering scaffolds (65,66). The controlled release of growth factors from such scaffolds could be important to enhance cell migration, proliferation, and differentiation. In order to prepare porous protein-loaded polymer scaffolds, prefabricated PEG/PBT scaffolds were used, of which the inner scaffold pores were coated with a protein-loaded polymeric film (67). To create the coating, a w/o emulsion was conducted through the pores of the scaffold by applying a vacuum. After freeze-drying, the resulting scaffolds contained a 10–40 μm coating, distributed over the scaffold pores. The release of a model protein (lysozyme) could be tailored by the emulsion composition. Figure 16 plots the release of a model protein, lysozyme, against time for three different w/p ratios. By decreasing

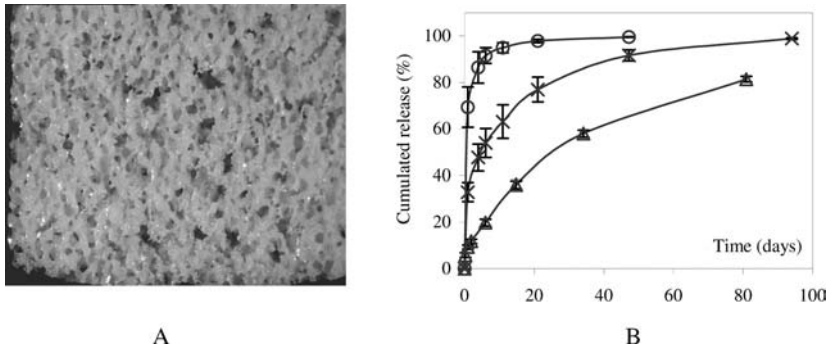

Figure 16 Light micrograph of a coated PEG/PBT scaffold (A) and lyozyme release from coated scaffolds (B). Coating were prepared from emulsions varying in ratio of aueous protein solution to polymer (⧗: w/p = 2, ○: w/p = 1, Δ: w/p = 0.5). (From Ref. 65.)

the w/p ratios, the release rates could be varied from a complete release within a couple of days up to over 2 months. No extensive decrease of the activity of the protein was observed (66). However, in future studies, the effectiveness of this protein release system should be assessed using relevant growth factors like rhBMP-2 or TGF-β.

5. THE STABILITY OF HYDROGEL-ASSOCIATED PHARMACEUTICAL PROTEINS

Protein degradation reactions can be divided into two broad classes. Chemical degradation reactions occur because the amino acids forming the primary structure of the protein are liable to oxidation, deamidation, isomerization, racemization, and disulfide exchange reactions. Peptide bond hydrolysis can occur as well (68,69). Some amino acids are known to be sensitive to particular degradation reactions. For example, cysteine, methionine, tryptophan, and tyrosine are readily oxidized. Deamidation reactions occur with asparagine and glutamine. Peptide bond hydrolysis often occurs around asparagine or aspartic acid residues.

Preservation of the secondary, tertiary, and—where relevant—quaternary structures of proteins is crucial for the pharmacological activity of the molecule. Both the therapeutic and the toxic effects exerted by the protein in vivo depend on these structural characteristics. These higher-order structures are mainly stabilized by physical forces such as electrostatic interactions, hydrogen bonds, hydrophobic interactions, and van der Waals interactions. In general, these forces are much weaker than covalent forces and, therefore, easily disturbed by changes in environment like temperature, pH,shear, and ionic strength. The molecular structure itself may change, and aggregation of colliding molecules may occuras well.

It is difficult to ensure full stability of a pharmaceutical protein both on the shelf and after administration and also before its release from the carrier system, after which release the protein in question becomes pharmacologically active. An illustration of this point will be helpful: an Ig molecule from

the IgG class has a molecular weight of 150 kDa and consists of over 1000 amino acids and of two sugar chains. Establishing the amino acid sequence and the chemical structure of all building blocks requires sophisticated modern analytical techniques like mass spectrometry. But the real and critical challenge is the assessment of the physical structure of the molecule. Our "toolbox" of physicochemical approaches with which to assess and monitor the higher-order structures is filled with chromatographic approaches, light scattering analyses, nuclear magnetic resonance (NMR) spectroscopy, x-ray crystallography, and different types of other spectroscopic approaches, ultracentrifugation, and calorimetric analyses (70). Immunoassays can also be used to assess structural aspects of proteins. And, finally, bioassays in vitro (cell systems) and in vivo (animal models) provide, of course, highly relevant information on a therapeutic or toxic effect of the molecule. But, in general, bioassays lack the sensitivity that is needed to pick up smaller changes in effect.

When monitoring the protein performance with all these techniques, the question arises which changes are relevant and which are irrelevant. Is *x*% deamidation or *y*% oxidation acceptable? Endogenous proteins secreted by the patients themselves, for example, are also present in different isoforms (in case of glycoproteins) or in partly degraded forms. Another issue is the sensitivity of proteins for degradation. Some proteins (e.g., lysozyme and albumin) tend to be rather robust. Interestingly, these compounds are rather often found as model proteins in stability studies. Other proteins are much more sensitive to external influences such as rhIL-2, growth hormones, and some interferons. In the discussion concerning acceptable and non-acceptable protein degradation, the potential immunogenicity of the therapeutic protein is of importance, as serious side effects have been ascribed to immune responses following the administration of pharmaceutical proteins (71). Aggregation is often seen as a major driver of immune responses, and serious efforts have been made to exclude aggregate formation in pharmaceutical formulations.

In light of the above findings, it is important, when considering protein-containing hydrogels as parenteral

controlled-release systems, to pay attention to protein stability. Surprisingly, in the existing literature, generally only one or a few analytical approaches were used from the "toolbox" for monitoring protein integrity. A study of the stability of IL-2 in hydrogels based on modified dex was published by Cadée and colleagues (72). These protein-loaded hydrogels were prepared without exposure to organic solvents ("all-water-hydrogel-formation"; see also Fig. 4 and Sec. 3.2). Crosslinking was established by radical polymerization with potassium peroxodisulfate (KPS) and TEMED as catalyst and initiator, respectively. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), high-pressure liquid chromatography (HPLC), and bioassays were used to monitor protein integrity. Also found were oxidized protein and some degree of aggregation, both under reduced and non-reduced conditions in the SDS-PAGE test. The released product still retained 60–70% of the bioactivity of the original product. In a second study, Cadée and colleagues (73) investigated in great detail the oxidation reactions of human IL-2 in the presence of KPS and TEMED. Here SDS-PAGE, reversed-phase HPLC (RP-HPLC), and mass spectrometry were the major analytical techniques for the monitoring of protein. If KPS alone was used, oxidation occurred in the methionines in IL-2 but not in the (buried) tryptophan. The presence of TEMED or other reducing agents clearly reduced oxidative damage, but some dimer formation occurred. In conclusion, oxidative damage by KPS can be modulated by adding other excipients.

Woo and colleagues (74) worked with composite microsphere systems based on both hydrophobic PLGA and the hydrophilic acrylic ester of hydroxyethyl starch (acHES). Albumin or horseradish peroxidase (HRP) was encapsulated in the acHES core particles, which were pre-crosslinked with KPS and TEMED. These protein-loaded core particles were later taken up in the PLGA microspheres. This idea of encapsulating the hydrogel acHES particulates in the hydrophobic PLGA microspheres arose from the notion that the current, standard PLGA microsphere technology, based on w/o/w double emulsion formation, does not properly protect

protein integrity because the protein is in intimate contact with the hydrophobic PLGA matrix. Sodium dodecyl sulfate polyacrylamide gel electrophoresis, size-exclusion chromatography/high-pressure liquid chromatography (SEC-HPLC), and enzyme activity (HRP) were used to monitor the fate of the protein. Protein material extracted from the microspheres showed no degradation with the chromatographic and electrophoresis techniques used. In the composite spheres, HRP enzyme activity was preserved for about 80%; for the conventional PLGA spheres, the activity dropped to about 60%. For both the PLGA/acHES and the PLGA microspheres, release patterns were very similar, showing large burst effects. Earlier, Wang and colleagues (75) explored a different route for the preservation of protein integrity in PLGA microspheres. They pre-encapsulated the model protein BSA in polyvinyl alcohol (PVA) microspheres and then dispersed these PVA spheres into PLGA microspheres. The authors conclude that the BSA was neither degraded nor aggregated. That may be true, but BSA integrity was monitored by SEC-HPLC only, and the validation of the analytical method was not reported. Recombinant interferon-α-2a (IFN-α) is one of the therapeutic proteins that has to be administered for long periods of time; it is a prime candidate for encapsulation in sustained-release systems. But, IFN-α is a tough candidate as it is a molecule that readily loses its structure. Zhou and colleagues (76) coated calcium alginate microcores containing IFN-α that had a poly-DL-lactide-poly(ethylene glycol) (PELA) film. The integrity of the protein was followed only by a monitoring of the biological activity, not by other means. Biological activity for IFN-α in the microspheres ranged from 6.5% in standard PLGA microspheres prepared via the o/w/o emulsion technique to 48% for the microcore-coated PELA microspheres. The authors indicate that further studies are under way to improve the performance of their composite systems.

Another approach for the minimization of the degradation of the protein during PLGA microsphere preparation and in the release phase is as follows: just mix PLA with PEG instead of water and make suspended BSA-containing

microspheres. The hypothesis is that the hydrophilic PEG acts as an early pore former, avoiding unwanted pH drops and protein degradation during disintegration of the microspheres (77). Structural information on the released BSA was obtained through SDS-PAGE and isoelectric focusing (IEF) electrophoretic, circular dichroism (CD) spectrometric, and fluorescence spectrometric analyses. The PEG content of the microspheres was varied between 0 and 30%. At the 10% PEG level, aggregates were observed, both non-covalently bonded and disulfide bonded. With 20% PEG, no aggregation could be observed anymore. The authors conclude that this reduction in BSA aggregation behavior is the result of an increased water content of the PEG-containing PLA spheres during the release process.

A different approach to make PLGA more "protein-friendly" was proposed by Kissel and collaborators (78,79). They synthesized block copolymer of poly(L-lactic-co-glycolic acid) (LPLG) and polyethyleneoxide (PEO) (LPLG-PEO-LPLG) linear triblock copolymers for the encapsulation of erythropoietin (EPO) in microspheres. Interestingly, the occurrence of covalently bound aggregates (20% as measured by SDS-PAGE) tended to be higher for the LPLG-PEO-LPLG than for the PLGA microspheres prepared according to the same protocol. Co-encapsulation of protective additives reduced the aggregation level to less than 5%. AB star-branched block copolymers consisting of A blocks of PLG or PLGA and star-branched PEG were synthesized as well. Sodium dodecyl sulfate polyacrylamide gel electrophoresis showed aggregation levels between 3 and 10%, levels that are much higher than those found in marketed regular EPO formulations (80). So far, this approach has not been met with much success.

An interesting case study was published by Van Dijkhuizen-Radersma and colleagues (58). Poly(ether-ester)s composed of hydrophilic PEGT blocks and hydrophobic PBT blocks were used to develop a sustained release system for calcitonin. Calcitonin is a relatively small protein that readily forms aggregates, often fibrillar in shape, in aqueous media. To avoid this aggregation, a number of additives were

screened with little success: calcitonin tends to form aggregates in the polymer and in solution, sooner or later. The calcitonin structure in the polymer in the dry solid state and upon hydration was monitored by Fourier transform infrared (FTIR). Interestingly, intermolecular beta sheets, suggesting non-covalent aggregation, could be observed both upon hydration of the hydrogel-forming polymer and before release of the protein from the hydrogel. This is one of the few examples where molecular properties of proteins inside the hydrogel polymer in the dry and hydrated states were established. Another example can be found in a short article by Van de Weert and colleagues (81) in which a description is given of the model protein lysozyme as it was monitored in PEGT/PBT films by FTIR. Similar non-covalently bonded aggregates were found in the films; but, when the protein was released, it was in its monomeric form, with full enzymatic activity.

6. CONCLUDING REMARKS

There is a need for reliable and versatile controlled-release systems for pharmaceutically active proteins. Hydrogel technology might meet these demands. This chapter demonstrates that the release profiles of proteins can be tailored by the hydrogel network structure. Importantly, both chemically and physically crosslinked hydrogels can be rendered biodegradable through the introduction of hydrolytically sensitive groups into the network. A major concern in protein carrier development is the integrity of the biopharmaceutical upon release. So far, protein-structure monitoring has only rarely been done, and if it is done, only a few groups make a real effort to collect basic information on protein degradation processes and ways to maintain integrity. However, from the information presently available, it is clear that hydrogels are, in terms of protecting the structure of the encapsulated protein, superior to alternative systems, like those based on hydrophobic polyesters. Although a number of attractive hydrogel systems are presently available, there is certainly room for novel systems with improved characteristics

methods. It is expected that principles from the expanding research area of supramolecular chemistry will be applied to the design of novel types of hydrogels with tailored properties. Also, protein engineering might contribute to the development of hydrogel systems with a very precise control over their microstructure.

Abbreviations

acHES:	acrylic ester of hydroxyethyl starch
BD:	1,4-butanediol
BSA:	bovine serum albumin
CD:	circular dichroism
dex:	dextran
dex-HEMA:	dextran derivatized with HEMA
dex-lactate-HEMA:	dextran derivatized with HEMA-lactate
dex-MA:	dextran derivatized with methacrylic acid
DMT:	dimethyl terephthalate
DP:	degree of polymerization
DS:	degree of substitution (number of methacrylate groups or oligo lactate grafts per 100 glucopyranose residues)
EPO:	erythropoietin
FTIR:	Fourier transform infrared
HEMA:	hydroxyethylmethacrylate
HRP:	horseradish peroxidase
IEF:	isoelectric focusing
IFN-α:	interferon-α-2a
IgG:	immunoglobulin G
KPS:	potassium peroxodisulfate
LPLG-PEO-LPLG:	block copolymer of poly(L-lactic-co-glycolic acid) and polyethyleneoxide
PBS:	phosphate buffered saline
PBT:	poly(butylene terephthalate)
PEG:	poly(ethylene glycol)
PEGT:	poly(ethylene glycol)-terephthalate
PELA:	poly-DL-lactide-poly(ethylene glycol)
PLGA:	poly(lactic-co-glycolic acid)
PVA:	polyvinyl alcohol
IL-2:	interleukin-2

rhIL2:	recombinant human interleukin-2
SEC-HPLC:	size-exclusion chromatography/high-pressure liquid chromatography
SL2:	Sutton lymphoma 2
TEMED:	*N*,*N*,*N*′,*N*′-tetramethylethylenediamine
w/o/w:	water-in-oil-in water
w/p:	water/polymer ratio

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6

Thermosensitive Biodegradable Hydrogels for the Delivery of Therapeutic Agents

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1. INTRODUCTION

Polymeric drug delivery is the most widely studied area of drug delivery in recent years (1). Polymers can be manipulated to possess certain properties that can meet specific criteria for the designing of suitable delivery systems. Polymeric drug delivery systems may provide advantages such as (i) increased efficacy, (ii) reduced side effects and toxicity, and (iii) convenience (1). Like small drugs in general, therapeutic macromolecular drugs require the use of polymeric systems, since these agents have very short half-lives in blood

plasma and are susceptible to physical or chemical degradation. Although many therapeutic proteins, peptides, and DNA-based drugs are available due to advances in biotechnology, the conventional routes by which these biotechnology-derived drugs are administered—routes such as intravenous injection, intravenous infusion, subcutaneous injection, and so on—require frequent injections to achieve a therapeutic concentration in blood and may result in poor patient compliance stemming from frequent, painful injections as well as undesirable side effects. Significant research effort has been made to develop implantable or injectable parenteral devices for the sustained and controlled release of protein drugs in order to reduce the frequency of injection (1).

Substantial efforts have been made to develop injectable polymeric drug delivery systems. Because many biomedical polymers with molecular weights of pharmaceutical interest are not water-soluble, certain solvents under a given toxicity limit may be used to dissolve the polymer. For example, Kost and coworkers studied polymer implants of poly(lactide-co-glycolide) (PLGA) dissolved in a water miscible solvent, glycofurol. When a subcutaneous injection of the drug-polymer-solvent mixture is made, this solvent diffuses into the bloodstream so that its concentration in blood on a per day basis is under the FDA limit (2). Another example is poly(ortho-ester) semisolid formulation (3). These systems use mild conditions and are suitable for protein delivery.

Polymers that exhibit physicochemical responses to stimuli have been widely explored as potential drug delivery systems. Kinds of stimuli investigated to date include chemical substances and changes in temperature, pH, and electric fields, and the like. Among these, polymers that, in an aqueous solution, exhibit dramatic changes upon a temperature change below or above the body temperature are of particular interest in drug delivery. For instance, low molecular weight triblock copolymers of PLGA and poly(ethylene glycol) (PEG) have been designed that are hydrophilic/hydrophobic balanced polymers. Jeong and coworkers first demonstrated a temperature-induced sol-gel transition of a PEG-PLGA-PEG aqueous solution upon subcutaneous injection in rats (4,5). More

recently, the intratumoral injection of Oncogel™ (Macromed, Inc., Sandy, UT), an injectable formulation of PLGA-PEG-PLGA (ReGel™) and paclitaxel, has demonstrated both the solubilization of a water-insoluble drug and the slow clearance of paclitaxel from the injection site with minimal distribution into other organs (6). This system is currently undergoing Phase II human clinical trials.

The use of thermosensitive polymers in drug delivery and biomedical application is widespread. The application of poly(*N*-isopropylacrylamide) [poly(NIPAAm)] and its copolymers is probably the best example. As mentioned above, thermosensitive and biodegradable polymers are becoming very important in the development of nontoxic, injectable systems to tackle challenging problems in the delivery of bioactive agents, as will be discussed in this chapter.

2. POLY(*N*-ISOPROPYLACRYLAMIDE) AND ITS COPOLYMERS

In the past two decades, studies have been carried out by a number of research groups to design polymers in aqueous environments that respond dramatically around certain transition temperatures (7–24). There are polymers that exhibit lower critical solution temperatures (LCST) in water, above which the polymer is insoluble. Poly(NIPAAm) has been of particular interest since the polymer shows an LCST of $\sim$32°C, which falls in the temperature range between body and room temperatures. Below the LCST, the enthalpy term contributed by the hydrogen bond between water and polar groups of the polymer causes the polymer to stay dissolved in water. Above the LCST, hydrophobic interaction, which is an entropic term, becomes a major factor that triggers the collapse of the polymers (7). The LCST of poly(NIPAAm) can be controlled via copolymerization with monomers having different degrees of hydrophobicity. It is known that a higher LCST can be obtained by incorporating a hydrophilic monomer, whereas copolymerizing with a hydrophobic monomer results in a lower LCST (7).

Han and Bae have demonstrated that an aqueous solution of a copolymer of 2–5 mol% acrylic acid and NIPAAm exhibits reversible gelation without significant hysteresis (8). The critical gel concentration (CGC) in this case was 4% by weight. Gelation, rather than precipitation, was attributed to polymer chain entanglements and to the weak physical association of polymer precipitates while hydration was maintained by an ionized segment of AAc (7,8).

Bae et al. have demonstrated the on/off thermo-control of solute release from poly(NIPAAm) networks (9,10). The swelling in water as a function of temperature for two series of NIPAAm polymer networks was investigated. In the first series, *n*-butylmethacrylate (BMA) was copolymerized with NIPAAm, and in the second, polytetramethylene ether glycol (PTMEG) was incorporated into a NIPAAm network as a chemically independent interpenetrating network (IPN). When an increase in the BMA content in the poly(NIPAAm-co-BMA) network occurred, a lowering of the gel collapse point was observed, and the gel's deswelling occurred in a more gradual manner as the temperature increased. The temperature dependence of equilibrium swelling in water was a function of the gel composition in both networks. The networks formed a dense skin layer as the temperature increased past the gel collapse point. This dense layer decreased the water efflux and formed water pockets at the surface (9).

In the next study poly(NIPAAm)/PTMEG IPNs were synthesized, and their feasibility as thermosensitive hydrogels for controlled drug release was addressed (10). The release of indomethacin incorporated into these matrices showed pulsatile patterns in response to temperature changes with sensitivity to a few degrees of fluctuation. The lag time and the release profile of indomethacin in the low-temperature region of each temperature cycle were influenced by the applied temperature and the gel composition. The results of this study demonstrated that solute release can be regulated by a rapid deswelling of the gel surface in response to an applied temperature change (10).

The unique solution behavior of poly(NIPAAm) has opened up a new array of applications. Hoffman and coworkers

developed methods for biorecognition and immunoassay using a series of poly(NIPAAm) polymers (11–14). In particular, poly(NIPAAm) was conjugated to a monoclonal antibody (MAb), and a novel separation method for an immunoassay was designed (11). The poly(NIPAAm) precipitated out of water above an LCST of 31°C, enabling a polymer-bound immune complex to be separated from the solution. The principal advantages of this method are that it utilizes a homogeneous incubation for the antigen-antibody reaction and that it has the ability to assay large-molecular-weight antigens with sensitivities equivalent to other nonisotopic heterogeneous immunoassays. In addition, because the polymer-immune complex may be reversibly redissolved by cooling, the method may be used both to concentrate the signal and to isolate the target analyte (11).

Okano and coworkers have also investigated extensively the phase transition behavior of poly(NIPAAm) hydrogel (15–17) and its applications in tissue engineering (16) and bioseparation (17). Takei and coworkers have demonstrated reversible bioseparation based on a similar concept. Immunoglobulin G (IgG) was modified by poly(NIPAAm) to create a novel bioconjugate that exhibits reversible phase transition behavior at 32°C in aqueous media. A terminal carboxyl group introduced into PIPAAm molecules by the polymerization of IPAAm with 3-mercaptopropionic acid was used for the conjugation to IgG via a coupling reaction of activated ester with a protein amino group. These conjugates exhibited a rapid response to changes in solution temperature and significant phase separation above the LCST corresponding to that for the original poly(NIPAAm). These conjugates bound to antigen quantitatively in the aqueous system, and the antigen-bound complex also demonstrated phase separation and precipitation above a critical temperature, indicating that PIPAAm conjugated to a biomolecule can operate as a switching molecule (17).

Feil et al. have demonstrated the molecular separation of solutes of three different sizes by using a poly(NIPAAm-co-butyl methacrylate)(BMA) copolymer hydrogel membrane at several temperatures (18,19). The diffusion of urinine (MW 300) and dextran (MW 4400) was found to follow the

free-volume theory in this hydrogel membrane although the partitioning of the solutes did not follow gel hydration due to partial exclusion and interactions between the hydrogel and the solutes. The screening effect of the polymer network played no major role for the diffusion of these solutes, but probably contributed significantly to the very low diffusion rate of dextran (MW 150,000) in the gel. Upon incorporating a pH-sensitive component (diethylaminoethyl methacrylate) they also found a mutual influence of temperature and pH on the swelling of the gel (18,19).

The copolymerization of NIPAAm with BMA and AAc rendered the copolymer pH sensitive as well as thermosensitive. This resulted in the design of copolymer beads as carriers for oral delivery, encapsulating polypeptide drugs such as calcitonin (20) and insulin (21). The copolymer beads prevent gastric degradation in the stomach while providing for a controlled release of a peptide drug later in the intestines. Serres et al. used linear terpolymers poly(NIPAAm-co-BMA-co-AAc) to fabricate beads loaded with a peptide drug, human calcitonin, which was dissolved in an aqueous phase. The polymeric beads were formed by the dropwise addition of a cold, aqueous solution of a temperature-sensitive polymer with human calcitonin into an oil bath kept at a temperature above the LCST of a polymer, precipitating the polymer and entrapping the peptide. The loading and the release of human calcitonin were also studied as a function of acrylic acid content in the terpolymers. As the acrylic acid content increased from 0 to 10 mol%, the loading efficiency and stability of calcitonin improved significantly. The same trend was observed for the quantity of released calcitonin. In vivo biological activity of the released hormone was preserved (20).

Taking a step further, Kim et al. have studied the loading, release, and the preservation of bioactivity of a polypeptide, insulin, entrapped in a microbead formation. The morphology of the beads studied by scanning electron microscopy (SEM) revealed that they consisted of a dense skin layer and a porous inner structure. This suggested that the critical step of bead preparation is the formation of this skin layer or

"surface curing." The release of insulin was triggered by a change in pH from acidic to neutral pH, whereas little release occurred at low pH. The released insulin had a preserved secondary structure as shown by CD spectroscopy. Also, it had preserved bioactivity as the injection of released insulin in rats showed no difference from standard insulin (21).

Ramkissoon-Ganorkar et al. have designed a novel polymeric delivery system that utilizes linear, pH/temperature-sensitive terpolymers of NIPAAm, BMA, and AAc. The unique properties of the pH/temperature-sensitive polymeric beads make it a potential system for the oral drug delivery of peptide and protein drugs to different regions of the intestinal tract (22). The goal of this study was to investigate the effect of polydispersity and of the molecular weight (MW) of terpolymers of poly(NIPAAm-co-BMA-co-AA) with a feed mol ratio of NIPAAm/BMA/AA 85/5/10 on the polymer dissolution rate and on the release kinetics of a model protein, insulin. Varying the average molecular weight (Mw) and polydispersity of the polymer modulated the dissolution rate of the polymer and the release rate of insulin from pH/ temperature-sensitive polymeric beads. An increase in the polydispersity of the polymer through the addition of high MW polymer chains caused a decrease in the release rate of insulin and in the polymer dissolution rate. High MW polymer chains impose a certain degree of interaction between polymer chains, and this is due to chain entanglement. There is a limiting value of MW above which chain entanglement has no effect on the drug release rate (22).

As mentioned earlier, poly(NIPAAm-co-AAc) where AAc content is small (2–5 mol%) forms loose hydrogels above the LCST. This makes the system an attractive candidate for tissue engineering application as an extracellular matrix (ECM). Above a critical concentration, aqueous polymer solutions of *N*-isopropylacrylamide copolymers with small amounts of acrylic acid, synthesized in benzene by free radical polymerization, exhibited four distinct phases as the temperature increased: a clear solution, an opaque solution, a gel, and a shrunken gel. The transition between the opaque solution phase and the gel phase was in the range of 30–34°C and was

reversible without syneresis and noticeable hysteresis under the experimental conditions used. Islets of Langerhans, isolated from a Sprague-Dawley rat pancreas and entrapped in the gel matrix, remained viable, with no significant decrease in the insulin secretion function in vitro for 1 month. When islets were encapsulated with the gel matrix in hollow fibers with a molecular weight cut-off (MWCO) of approximately 400,000 and were exposed to dynamic changes in glucose and theophylline concentrations, their insulin secretion patterns exhibited a smaller lag time and higher amplitude in insulin release than did the islets entrapped in a conventional alginate matrix under the same experimental conditions. From these two observations, that is, the gel reversibility and the islet functionality in the matrix observed in the in vitro experiments, the *N*-isopropylacrylamide copolymers with acrylic acid synthesized in this study seem to be optimum candidates for the ECM for the recharging of the entrapped cells when the decrease in the cell functionality in the system is noted (23).

The purpose of a second application of poly(NIPAAm) in tissue engineering concerns the grafting of poly(NIPAAm) onto the cell culture surface so that cells can both attach under the growing condition (above the LCST) and easily and reversibly detach below the LCST. This is important in that the conventional method of cell detachment using proteolytic enzymes can damage cells. This concept brought about the so-called "cell sheet engineering" in which this type of surface is used. Such technology may be useful in the repairing of damages in various organs (24).

3. PEO-PPO-PEO TRIBLOCK COPOLYMERS

Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) is another kind of thermosensitive polymer, known as poloxamer (25,26). Poloxamers are a series of surfactant polymers that are commercially available. Among these, poloxamer 407 (Pluronic F127) has been of particular interest, since this amphiphilic triblock copolymer can form micelles at low concentration in water and, with increasing concentration, can form a hydrogel at body temperature via

packing of the micelles. This means that the aqueous solution of this polymer may be used for a parenteral depot system. However, the gel depot of the PEO-PPO-PEO block copolymers disintegrated quickly (within 1 day) upon dilution and therefore is not suitable as a sustained release system. It was speculated that this surface dissolution may be related to its gelling mechanism, which involved the mere packing of micelles at high concentrations (25).

4. THERMOSENSITIVE AND BIODEGRADABLE POLYMER HYDROGELS

Biodegradable polymers used for drug delivery to date have mostly been in the form of injectable microspheres or implant systems, which require complicated fabrication processes using organic solvents. A disadvantage of such systems is that the use of organic solvents can cause denaturation when protein drugs are to be encapsulated. Furthermore, the solid form requires surgical insertion, which often results in tissue irritation and damage.

Synthesis of a thermosensitive, biodegradable hydrogel consisting of blocks of poly(ethylene oxide) and poly(L-lactic acid) was carried out (4). Aqueous solutions of these copolymers exhibited temperature-dependent reversible gel-sol transitions. The hydrogel can be loaded with bioactive molecules in an aqueous phase at an elevated temperature (around 45°C), where they form a solution. In this form, the polymer is injectable. On subcutaneous injection and subsequent rapid cooling to body temperature, the loaded copolymer forms a gel that can act as a sustained release matrix for drugs (4).

In the next series of studies, Jeong et al. found compositions of HPL/HPB balanced triblock copolymers, PEG-PLGA-PEG, that exhibit sol-to-gel (lower transition) and gel-to-sol (upper transition) transitions as temperature monotonically increases (27). The lower transition is important for drug delivery application because the solution both flows freely at room temperature and becomes a gel at body

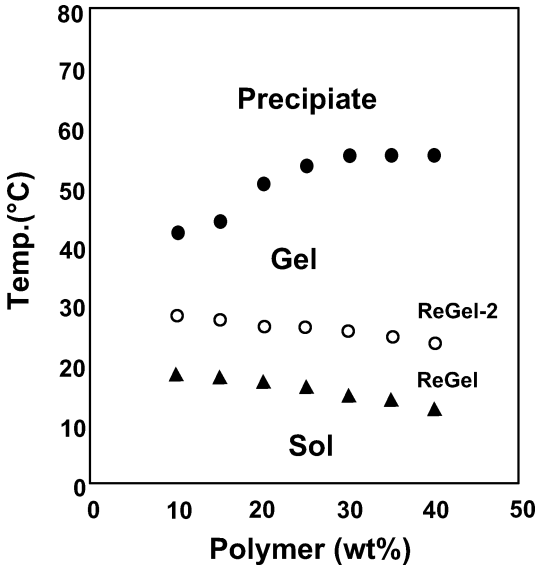

Figure 1 Phase diagram for PLGA-PEG-PLGA in aqueous solution (ReGel^{TM}). (From Refs. 6.)

temperature. The lower transition may be related to micellar growth and intra- and intermicelle phase mixing and packing, while the upper transition involves a breakage of the micellar structure. The CGC and critical gel temperature are controlled by polymer parameters, such as block length and composition of PEG-PLGA-PEG triblock copolymers, and by additives such as salts (27).

When aliquots of the 33 wt.% aqueous solutions of PEG-PLGA-PEG triblock copolymers were administered via subcutaneous injection into rats, transparent gels were observed. The gels showed good mechanical strength and the integrity of the gels persisted longer than 1 month. The gel underwent degradation by hydrolysis and became opaque, a development that was probably due to the preferential mass loss of PEG-rich segments from the in situ formed gel. The number-averaged molecular weight (Mn) determined by gel-

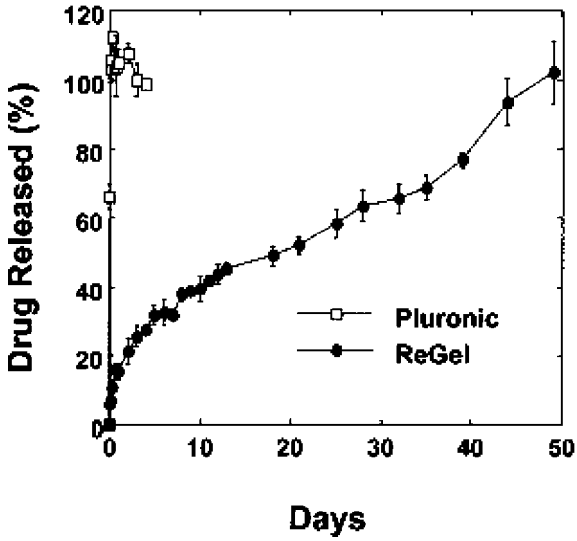

Figure 2 In vitro release of paclitaxel from OncoGel™ (23%, w/w in water) and Pluronic F-127. (From Ref. 6.)

permeation chromatography (GPC) decreased from 3300 to 1900 and a 30% mass loss was observed over 1 month (5).

Two model drugs were released from the PEG-PLGA-PEG triblock copolymer hydrogel formed in situ at 37°C. While ketoprofen, a model hydrophilic drug, was released over 2 weeks with a first-order release profile, spironolactone, a model hydrophobic drug, was released over 2 months with an S-shaped release profile. The release profiles were simulated by models considering both degradation and diffusion, and were better described by a model assuming a domain structure of the gel (28).

The ABA-type triblock copolymer, PLGA-PEG-PLGA, is also a biodegradable, biocompatible polymer that demonstrates reverse thermal gelation properties (6,29). Its phase diagram is shown in Figure 1. The unique characteristics of the gel (ReGel™, Macromed, Inc.) lie in the following two key properties: (i) the triblock copolymer is a water soluble, biodegradable polymer at temperatures below the gel transi-

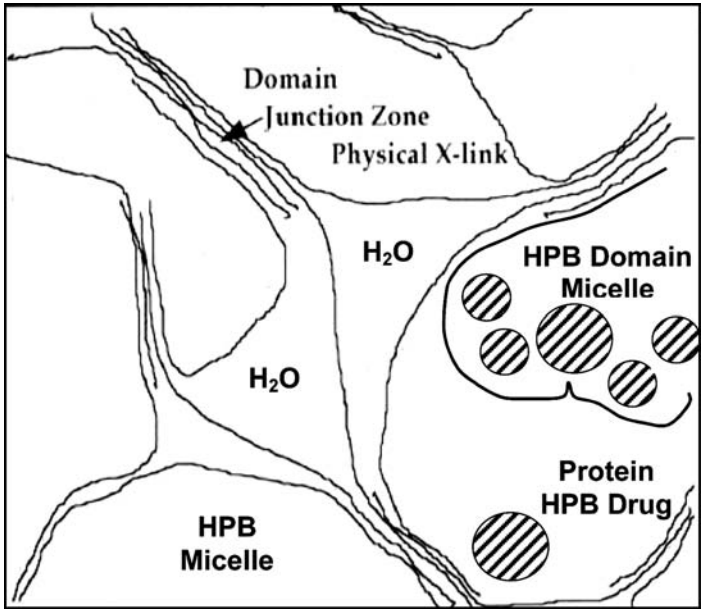

Figure 3 Physical crosslinks in the PLGA-PEG-PLGA in water (ReGel^{TM}) showing hydrophobic domain structures (Courtesy of Macromed, Inc., Sandy, UT.)

tion temperature; and (ii) the polymer also forms a water-insoluble gel once injected. This hydrogel formed above transition temperature possessed physical interactions between hydrophobic domains. A dynamic mechanical analysis revealed that an increase in viscosity of approximately 4 orders of magnitude accompanies the sol-gel transition. The gel forms a controlled release drug depot with delivery times ranging from 1 to 6 weeks. The inherent ability of the polymer to solubilize (400 to >2000-fold) and stabilize poorly soluble and sensitive drugs, including proteins, is a substantial benefit. The gel provided excellent control of the release of paclitaxel for approximately 50 days, as shown in Figure 2. Paclitaxel loaded in the hydrophobic domain (Fig. 3) slowly diffuses out in the first phase of release followed by a more rapid release when the degradation of the polymer matrix

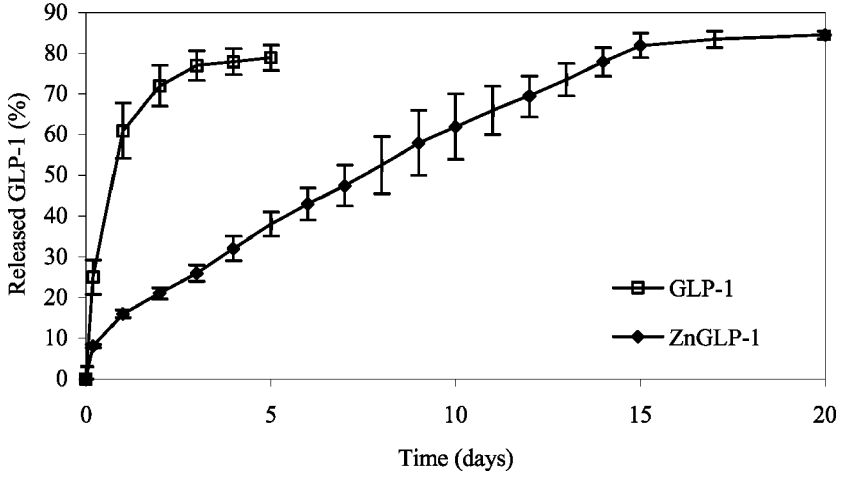

Figure 4 Released GLP-1 from ReGel^{TM} formulation in vitro. The graph represents the average ± SE, and each group was composed of three samples. (From Ref. 32.)

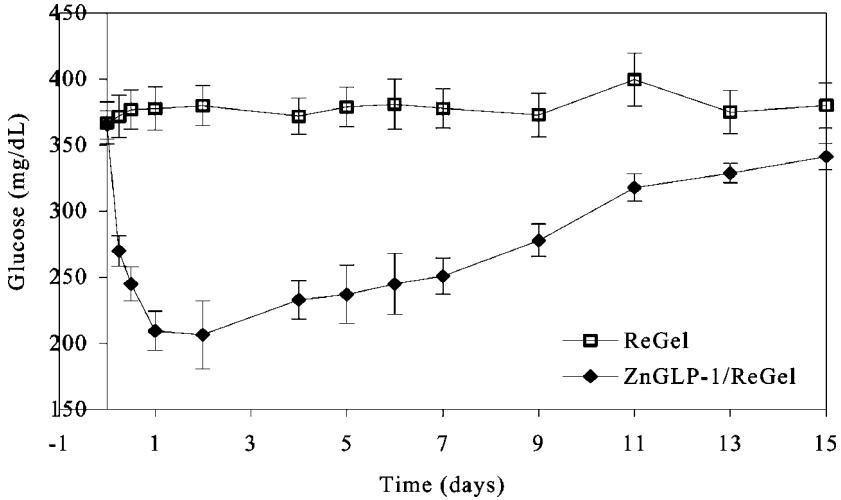

Figure 5 Blood glucose level in ZDF rats after injection. The graph represents the average ± SE, and each group was composed of five rats. (From Ref. 32.)

becomes more significant. Direct intratumoral injection of ReGel/paclitaxel (OncoGel^{TM}) was mentioned earlier. Efficacies equivalent to maximum tolerated systemic dosing were observed at OncoGel doses that were ten-fold lower (6).

This ABA-type triblock copolymer was used as a drug release depot for continuous release of human insulin and of glucagons-like peptide-1 (GLP-1). The observation of both reduced initial burst and a constant release of human insulin from ReGel in vitro is due to the domain structure of the gel and to the modification of the association states of insulin by zinc. Animal studies using SD rats were performed to verify the release profile of insulin from this ABA block copolymer hydrogel. ReGel formulation maintained insulin release for up to 15 days, which could allow diabetic patients to reduce the number of insulin injections to two per month for basal insulin requirements (31).

The triblock copolymer hydrogel was used also as an injectable formulation for the controlled release of GLP-1 in vitro and in vivo (32). Because the aqueous solution of ReGel used in this study has a sol-gel transition around 32°C, the mixture of the GLP-1 and the aqueous free-flowing polymer solution spontaneously formed a gel at body temperature. GLP-1 was formulated into ReGel as an insoluble zinc complex to stabilize GLP-1 against aggregation and to sustain the release rate. The in vitro GLP-1 release from ReGel formulation at 37°C showed no initial burst, as shown in Figure 4. Further release was controlled by zero order kinetics. An animal study using Zucker Diabetic Fatty (ZDF) rats, as a type-2 diabetes animal model, showed that a blood glucose level was maintained at mild hyperglycemic level as shown in Figure 5. The glucose level dropped to a level significantly lower (~200 mg/dL) than the control (~400 mg/dL). A single injection of ZnGLP-1 loaded ReGel can be used for the controlled delivery of bioactive GLP-1 over a 2-week period (32).

5. BIODEGRADABLE MICROSPHERES BASED ON THE THERMOSENSITIVE PROPERTY OF PLGA-PEG-PLGA

Injectable controlled release systems based on biodegradable copolymers of lactic and glycolic acids (PLGAs) have become widely used for the delivery of both protein therapeutics and vaccine antigens (33–47). Although numerous protein therapeutics have been approved or are in clinical trials, the development of more sophisticated delivery systems for this rapidly expanding class of therapeutic agents has not kept pace. The short in vivo half-lives, the physical and chemical instability, and the low oral bioavailability of proteins currently necessitate their administration by frequent injections of protein solutions. This problem can be overcome through the use of injectable depot formulations in which the protein is encapsulated in, and released slowly from, microspheres made of biodegradable polymers. Although the first report of the sustained release of a microencapsulated protein was more than 20 years ago, the instability of proteins in these dosage forms has prevented their clinical use. Advances in protein stabilization, however, have aided the development of sustained release forms of several therapeutic proteins, and clinical testing of a monthly formulation human growth hormone was carried out. The obvious advantage of this method of delivery is that the protein is administered less frequently, sometimes at lower overall doses, than when formulated
as a solution. More importantly, this method of delivery can justify commercial development of proteins that, for a variety of reasons, could not be marketed as solution formulations (36).

However, there are drawbacks observed in protein-loaded microspheres. The protein release kinetics exhibits an initial fast release followed by a slow release, resulting in an incomplete protein release despite significant degradation of microspheres (36). The very slow release kinetics was attributed to the protein aggregation and nonspecific adsorption within the microspheres. It was found that the protein

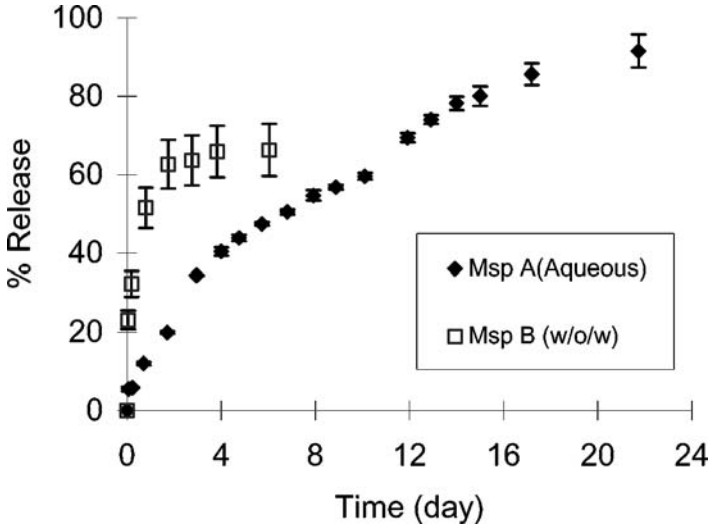

Figure 6 In vitro release of human insulin from Msp A (aqueous-based) and Msp B (dichloromethane-based) ($n = 3$, mean $\pm$ SD). (From Ref. 49.)

was significantly denatured and aggregated during the double emulsion formulation step (35). This problem is inherent in the fact that the polymer (e.g., PLGA) is not water soluble; thus a water-immiscible solvent (i.e., methylene chloride) has to be used. This also means that high mechanical energy input for the dispersal of the proteins in the organic phase and in this process w/o interface is created (37). Kwon et al. have observed that rapid aggregation of insulin is induced by the interface between water and methylene chloride (38).

For example, Nutropin^{TM} (Genentech, Inc., South San Francisco, CA), human growth hormone-loaded PLGA microspheres (40), was approved by FDA. However, the initial burst effect was not resolved. Burst release can result in acute overdosage and this could lead to fluid retention, headache, nausea, vomiting, or hyperglycemia.

Over the last few years, improvements were suggested toward overcoming the difficulty of stabilizing PLGA-

encapsulated proteins. In addition to the stabilization of proteins during encapsulation with anhydrous methods, protein complexation with zinc and the control of PLGA microclimate pH with basic excipients were also suggested (33).

One way to overcome the problems associated with both PLGA microspheres and the use of organic solvents is to utilize the thermosensitive property of PLGA-PEG-PLGA so that protein loading can be achieved without having to use organic solvents such as CH_2Cl_2, hence avoiding water/organic solvent interfaces (48,49).

Microspheres of the biodegradable, triblock copolymer (PLGA-PEG-PLGA, Mw = 4000, 1500–1000–1500 by NMR) were prepared in two methods: microsphere A (Msp A; aqueous-based) and microsphere B (Msp B; dichloromethane). For both microspheres, an equal amount of Zn-insulin was loaded (~4% of polymer mass). In vitro release studies were carried out with both. As shown in Fig. 6, Msp A exhibited a continuous and nearly complete release of insulin over 3 weeks. The first phase of insulin release (the first 10 days) from Msp A seems to be dependent more upon diffusion, indicated by the slight decrease in the release rate over time. Then, after day 10, the insulin release rate turned to an

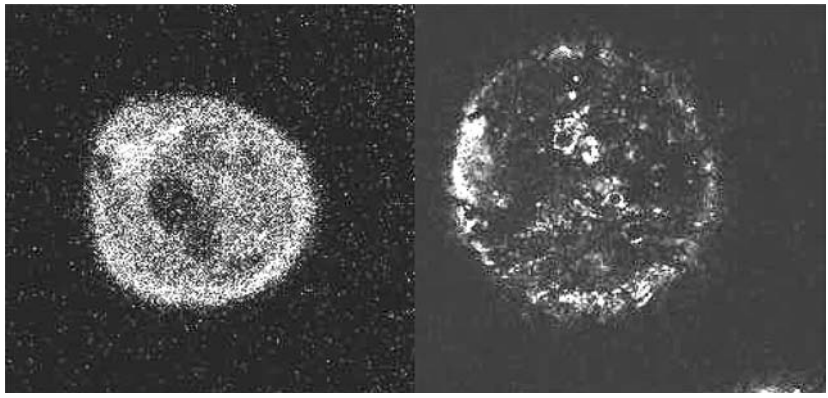

Figure 7 Confocal microscopy images of FITC-insulin loaded microspheres. (Left) Msp A; (right) Msp B. (From Ref. 49 with permission.)

increasing mode, and this is probably where the degradation of the matrix, at this time point, begins to play a more significant role in release than in the earlier phase. However, Msp B exhibited an initial burst release (~50% in 1 day), and release was discontinued at ~60% afterwards. In the preparation of Msp B, during the formation of primary emulsion where it involves high shear and heat generation in the creation of a large water/organic solvent interfacial area, proteins can undergo rapid aggregation, and thus the incomplete release of proteins from microspheres may be due to these trapped aggregates formed during microsphere fabrication. This accounts for the slow and incomplete release after the initial release phase with the burst effect (48).

In the case of Msp A, microspheres were prepared in a mild environment; that is, an organic solvent and a high shear were absent. A circular dichroism (CD) spectrum of insulin released from Msp A was virtually identical to that of a freshly prepared native insulin solution. This means that the released insulin preserved its secondary structure. In contrast, the CD spectrum for Msp B indicated a loss of secondary structure integrity due to both the use of dichloromethane and the harsh preparation conditions employed (48).

The observed release pattern from both types of microspheres lies in the distribution of the protein inside a microsphere, which is associated with the preparation method. In order to see this, FITC (fluorescein isothiocyanate)-insulin incorporated microspheres were observed under a confocal microscope, as shown in Fig. 7. For Msp A, a homogeneous distribution of fluorescence was observed while Msp B exhibited a rather heterogeneous distribution of FITC-insulin. In addition, Msp B shows significant surface fluorescence. These observations are, hence, consistent with the observed initial burst from Msp B and from the constant insulin release from Msp A over a prolonged period of time. It is reported that the constant release of insulin from triblock copolymer hydrogel may be attributed to the hydrophilic/hydrophobic domain structure of the gel. The incorporation of a significant fraction of insulin in the hydrophobic domain may have made possible

the sustained release of insulin (49).

An animal study with streptozotocin-induced diabetic rats was carried out and involved the subcutaneous injection of both microspheres. While Msp B caused a burst effect (hypoglycemia) followed by a quick change in blood glucose and insulin levels, Msp A exhibited relatively sustained blood glucose levels and the release of insulin for 10 days. In vitro and in vivo insulin release profiles were found to be rather consistent (49).

6. CONCLUSIONS

The utility of thermosensitive hydrogels in drug delivery and biomedical application is immense. In particular, biodegradable, thermosensitive triblock copolymers can be formulated into potentially useful drug delivery systems for therapeutic protein drugs or poorly water soluble drugs because such copolymers exhibit unique aqueous solution properties, biodegradability, and biocompatibility. Single injections of paclitaxel, insulin, and GLP-1 in the triblock copolymer hydrogel (ReGel) have demonstrated sustained and controlled release patterns of the agents both in vitro and in vivo. Based on the thermosensitive property of the triblock copolymer, PLGA-PEG-PLGA, biodegradable microspheres for protein delivery have been designed and continuous insulin release achieved, resulting in both a significant reduction in burst release and the preservation of bioactivity. These systems can be potentially useful for the delivery of a wide range of therapeutically challenging agents such as DNA as well as protein and water-insoluble/toxic drugs.

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7

Hydrogels

Stimuli-Sensitive Hydrogels

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1. INTRODUCTION

Hydrogels are physically or chemically cross-linked polymer networks swollen with large amounts of water. Due to their crosslinked nature, these gels do not dissolve in aqueous media but contain an enormous amount of solvated water molecules within the entangled polymer chain matrix. Hydrogel properties are reviewed elsewhere in this book. This chapter is dedicated to a unique hydrogel family that responds to

externally applied stimuli that, in turn, alter these hydrogels' swelling properties. During the past two decades, much work has been dedicated to the development of stimuli-responsive hydrogel materials. These "intelligent materials" sense external stimuli, alter (depending on the degree or strength of stimulation) their physicochemical network properties, and release drug molecules or absorb water or both to reach an equilibrated state. Such auto-feedback systems are commonly observed in metabolic processes in the human body. Therefore, these intelligent materials are appropriately applied to the development of new drug delivery matrices responding to several physiological stimuli arising from disease states or metabolic events in the human body. One key strategy for drug delivery systems is the spatio-temporal control of drug release responding to any changes in body physiology at specific sites (1,2).

In the present chapter, several types of stimuli-responsive hydrogels are introduced, and their applications to drug delivery systems will be reviewed.

2. STIMULI-RESPONSIVE HYDROGELS AND THEIR APPLICATIONS IN DRUG DELIVERY SYSTEMS

2.1. Temperature Sensitive Polymeric Materials

Several types of hydrogels are known to undergo physical changes in response to changes in temperature. These include poly(vinylmethyl ether)s cross-linked by gamma ray irradiation (3), poly(*N*-isopropylacrylamide) (PIPAAm) and its derivatives (4–15), poly(ethylene oxide)-*b*-poly(propylene oxide) -*b*-poly(ethylene oxide) triblock copolymers (16–19), poly(alkyl vinyl ether)s and their block copolymers, hydroxymethylcellulose, gelatin, and other more exotic materials. All of these materials share in common a unique hydration chemical structure in aqueous milieu that is metastable and can be radically altered by increasing the thermal energy in the system. Changing temperature therefore often produces a dramatic and pseudo first-order phase change resulting from

the dehydration and rehydration of the materials' chemistry, resulting in a collapse and expanding behavior in water. This phase transition is reversible, with some characteristic hysteresis, upon reversal of the temperature change. Many materials exhibit this property (20–23).

2.1.1. Poly(*N*-isopropylacrylamide) Hydrogels for Thermo-Responsive Drug Delivery

With several chemical features analogous to proteins, polyamides, and poly(amino acids), PIPAAm was synthesized as a model polypeptide analogue so that its solution behavior in water could be investigated (24). PIPAAm in an aqueous solution shows a lower critical solution temperature (LCST) at 32°C. This unique temperature response of PIPAAm in an aqueous solution has been extensively investigated for use in stimuli-responsive materials for biomedical applications in drug delivery systems, bioseparations, bioconjugates, and noninvasive cell manipulations (4–7,9,11,25–42). In cross-linked PIPAAm networks, the polymer's aqueous soluble-insoluble changes that occur in relation to temperature result in reversible swelling-deswelling in the hydrogels. Copolymerization of IPAAm with either hydrophilic or hydrophobic monomers produces an increase or decrease in the transition temperature of PIPAAm. Such a strategy can be exploited so that a hydrogel's transition temperature can be controlled and that the drug release behavior can, thus, be regulated. In the present section, recent advances in the development of thermo-responsive hydrogels and their applications in drug delivery systems are reviewed.

For PIPAAm hydrogels, a large temperature increase originating below the polymer transition temperature of 32°C induces an outside-in shrinking response in the gel: thermal transfer and polymer mass transfer kinetics compete for the determining of the polymer phase behavior. For this condition, the result is the formation of a dense shrunken collapsed polymer layer at the gel-water interface (a skin layer). These dehydrated polymer skin layers on the surface of shrunken PIPAAm hydrogels prevent even water molecules from

readily diffusing through the gel. Thus, drug release from PIPAAm and their derivative hydrogels is governed by a drug's bulk water diffusion below the transition temperature, with the release of the drug completely impeded above the transition temperature (5,6,31). This temperature-dependent drug release mechanism produces a thermo-responsive on-off drug release with PIPAAm hydrogels that is useful for triggered thermal release control.

Rapid swelling-deswelling gel kinetics cannot be obtained with conventional hydrogels in which mass transfer kinetics are governed by the reciprocal of the squared dimension of the gels (23). Therefore, both the inclusion of macro- or microporous structures within gels and a reduction in gel size are common methods by which to accelerate gel shrinking behavior (3,13,43). To overcome this limited swelling-deswelling kinetics issue, we have introduced freely mobile grafted PIPAAm chains within multiple bonded three-dimensional cross-linked PIPAAm hydrogels (10,44,45). Using telomerization polymerization, we prepared a chain transfer agent in the form of PIPAAm chains that had one terminal amino end group and that were in the presence of aminoethanethiol. Terminal amino groups were then converted, through a reaction with acryloyl chloride, to polymerizable acrylamide moieties. PIPAAm macromonomers were then copolymerized with an IPAAm monomer in the presence of the cross-linker, *N,N′*-methylenebisacrylamide. The structural formula is shown in Fig. 1. These novel graft-type PIPAAm hydrogels exhibit the same transition temperature as conventional PIPAAm hydrogels at 32°C but also exhibit highly altered swelling-deswelling kinetics. The molecular weight of the grafted PIPAAm chains showed significant influences on gel deswelling. Comb-type grafted PIPAAm hydrogels with different graft chain lengths were examined for their swelling-deswelling characteristics. These kinetics were investigated with PIPAAm hydrogels containing grafted side chains of 2900, 4000, and 9000 g/mol. PIPAAm grafted gels with a graft molecular weight of 9000 (IGG9000) showed rapid shrinking kinetics upon a temperature increase above the transition temperature in buffer at 32°C. Such rapid gel shrinking is

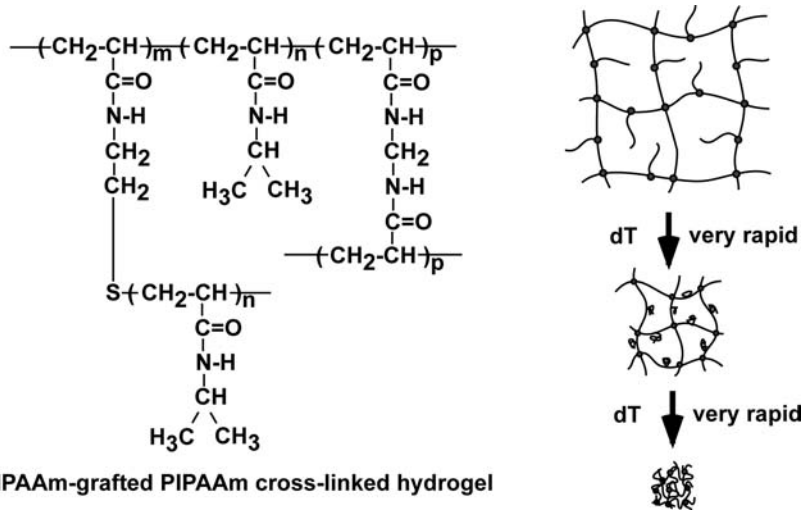


Figure 1 Structural formula of linear PIPAAm-grafted PIPAAm hydrogels and schematic illustration of their shrinking behavior.

probably due to the existence of grafted PIPAAm chains that, owing to their mobile nature in the gel, dehydrate and then aggregate upon a temperature increase prior to bulk gel dehydration. Such hydrophobic aggregation of grafted chains induces and accelerates the entire gel-deswelling behavior. However, hydrogels with grafted chains of lower molecular weight showed relatively slow deswelling, with only partial skin layer formation, even though the overall grafted chain content was the same (approximately 30 wt.%) as for the PIPAAm gels having longer grafted chains. The cylinder-shaped, faster deswelling hydrogel, IGG9000, showed repetitive and large oscillations in length when gels were subjected to temperature cycles between 20 and 40°C with hysteresis accorded to collapse-rehydration dynamics and water mass transport in these bulk systems (44).

Such differences in bulk gel-deswelling characteristics have a significant influence on the release of incorporated drug molecules, especially for higher molecular weight drugs. Figure 2 shows the temperature-induced drug release behavior from conventional and PIPAAm-grafted hydrogels that

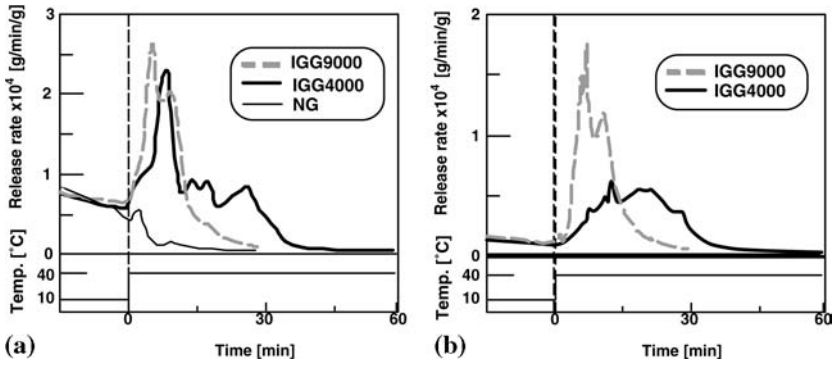


Figure 2 Thermo-responsive drug release behavior from PIPAAm-grafted PIPAAm hydrogels upon step temperature gradient for (a) sodium salicylate, and (b) dextran of MW 9300.

had different graft chain lengths. Figure 2a illustrates the release of small, water soluble sodium salicylate from these gels upon a temperature increase from 10 to 40°C. Drug release from conventional PIPAAm hydrogels was pulsed. Immediately after the temperature increased, a small amount of the drug was released, and then a substantial decrease in the amount of the drug being released took place so that the release was completely impeded after 15 min. This result is due to the impermeable, dense collapsed polymer skin layer formation at the gel's outer surface. Drug release from graft-type hydrogels exhibited rapid, pulsed release; 65% of drug molecules incorporated were released from IGG9000 within the first 15 min after temperature change. IGG4000, however, exhibited oscillating release profiles. For relatively lower molecular weight grafted chains, chain aggregation forces still operated within the gels above the transition temperature, and thus, surface skin layers were formed on these hydrogel surfaces. However, these lower molecular weight chains' collapse forces are weaker than those of longer chains: the increasing internal hydrostatic pressure resulted from the bulk gel shrinking of both the aggregated grafted chains and, subsequently, the main chain. These hydrostatic forces eventually overwhelm the weaker short-chain collapse forces on

the device surface, an outcome that allows a pulse of outward water-drug flux to release the pressure gradient. This produces a cyclic, pulsatile drug release profile.

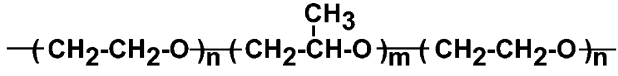
For higher molecular weight drugs (e.g., dextran MW 9300), a burst release was observed for IGG9000 hydrogels after a temperature increase to 40°C (Fig. 2b). By contrast, the dextran release from IGG4000 was relatively suppressed and distinctly different from the small molecule case. These differences in drug release behavior for the two grafted gels are probably due both to the chain aggregation forces operating within the hydrogels and to their influence on diffusing molecules of different size. That is, longer grafted chains form more hydrophobic cores within the hydrogels immediately after a temperature increase above the transition temperature, an outcome that facilitates faster hydrogel deswelling and hydrostatic pressure-driven outward transport. In contrast, the hydrophobic aggregation between the dehydrated, shrunken grafted chains of IGG4000 is comparatively relatively weak. Therefore, the thin collapsed polymer skin layers formed at the interface of IGG4000 gels limit and retard large drug diffusion from the gel's interior. These results strongly suggest that the drug release behavior of various drugs with a diverse range of molecular weights can be regulated with the grafted chain lengths of comb-type grafted PIPAAm hydrogels.

Similar fast deswelling characteristics were observed using poly(ethylene glycol) (PEG)-grafted PIPAAm hydrogels similar in structure to PIPAAm-grafted hydrogels above the characteristic transition temperature near 32°C (46). PEG-grafted chains did not alter the bulk hydrogels' thermoresponsive characteristics, although PEG itself is a highly hydrophilic polymer. Thus, hydrophilic PEG-based channels or pores remained after the bulk gel transition. This outcome is in sharp contrast to conventional PIPAAm hydrogels containing acrylamide (AAm) or acrylic acid (AAc) as the hydrophilic co-monomer, since the introduction of these co-monomers incorporates random compositional distribution throughout the bulk gel and is not polymer grafting per se. This hydrophilic monomer incorporation produces a

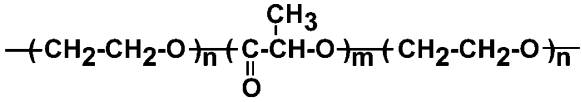
significant increase in the gel transition temperature, and further co-monomer introduction eventually abolishes the thermo-responsive characteristics of PIPAAm hydrogels. Maintenance of the thermo-responsive characteristics of PEG-grafted hydrogels is probably due to the structural independence of PEG-grafted chains and PIPAAm cross-linked chains. The deswelling mechanism for PEG-grafted PIPAAm hydrogels is distinct from PIPAAm-grafted hydrogels, as PEG chains are highly hydrated at all of the temperature ranges examined. The PEG chains form water-releasing channels within the dehydrated and collapsed skin layers on the shrinking PIPAAm hydrogels owing to a microphase separation of PEG chains within the collapsing PIPAAm phase. This effect accelerates bulk gel-deswelling above the transition temperature. Therefore, rapid hydrogel dehydration is achieved. Using this unique deswelling mechanism that shrinks PEG-grafted PIPAAm hydrogels, researchers can achieve both rapid drug release and bulk water release. Furthermore, such hydrogels can be used to target temporal drug releases, activated only when stepwise temperature stimuli are applied at target sites.

2.1.2. Thermo-Responsive Sol-to-Gel Transitions and Exploitation for Injectable Drug Delivery

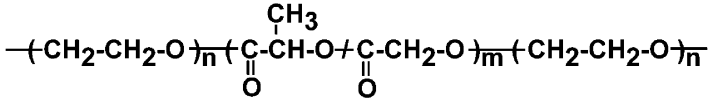
Poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (PEO-PPO-PEO) (Fig. 3) is a well-known thermo-responsive self-assembling polymer with the trade name of Pluronic®, (BASF, Florham, NJ., USA) (47). A variety of Pluronics have been developed with varying compositions of PEO and PPO blocks. Pluronics with relatively large PEO weight ratios are soluble in water through the self-association of relatively hydrophobic PPO segments, resulting in the formation of polymer micelles. These polymeric micelles exist in an equilibrated state between monomeric polymer molecules and micelles. Some types of Pluronics exhibit thermally reversible physical gelation at certain concentration ranges and temperatures (48). Pluronic F127, containing 70 wt.% PEO with



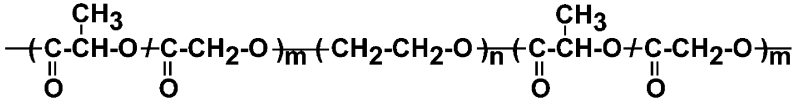
Pluronic; PEO-PPO-PEO



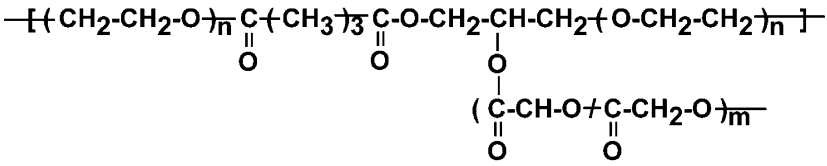
PEO-PLA-PEO



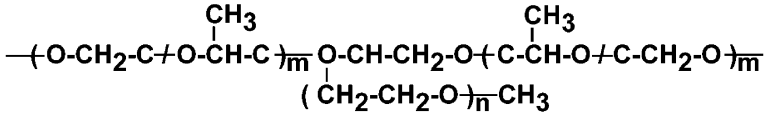
PEO-PLGA-PEO



Re-Gel ; PLGA-PEO-PLGA



PEG-*graft*-PLGA



PLGA-*graft*-PEG

Figure 3 Structural formulae for a variety of types of thermogelling materials.

12,000 MW triblock segments, is a typical example of a thermally reversible gelation material. Aqueous solutions of Pluronic F127 (>20 wt.%) gel spontaneously upon a temperature increase of ~20°C without syneresis of water molecules; that is, no volume change occurs during gelation. This outcome is due to the dense packing of polymeric micelles, which occupy the entire volume of the polymer solution and encapsulate all of the bulk water. Such gels achieve a thermally reversible solution state upon a decrease in temperature. This unique property has been utilized for injectable drug delivery systems, especially for labile drugs such as peptides and proteins. The Pluronic drug delivery formulation results from the mixing of polymers directly with a drug at low temperatures, followed by injection of the mixture into the body. At injection sites, the Pluronic solution—once placed in the body—gels owing to a temperature increase. Drug molecules then diffuse from the in situ-formed gels in a controlled release fashion (16,17,19,49,50). Pluronic gel monoliths slowly dissolve owing to their dilution with body fluids and are ultimately excreted from the body. Thus, drug release can be extended from several hours to a few days, with drug release for longer periods not achievable with this formulation.

Kim and his colleagues (51–55) designed ABA-type triblock polymers that, as do Pluronics, exhibit thermoresponsive gelation at body temperature. As this book has devoted one chapter for these thermogelling triblock copolymers and their applications in drug delivery systems, only limited information will be given here.

The researchers used methoxy-terminated PEG with a known molecular weight for macroinitiator, to initialize the ring-opening polymerization of lactide or glycolide and formed a PEG-polylactide diblock copolymer. Then, two diblock polymer molecules were coupled using hexamethylene diisocyanate to form PEG-PLLA-PEG (51,56) or PEG-PLGA-PEG (52,53,55) triblock copolymers that exhibited a relatively narrow molecular weight distribution ($Mw/Mn \sim 1.2$). As shown in Figure 3, these triblock copolymers contain biodegradable polyester segments. Researchers found the thermally induced gelation of triblock polymer aqueous solutions at 17–40 wt.%

near 30–36°C (52) (Fig. 4). A further increase in temperature affected gel appearance, from transparent to turbid (just above the physiological temperature), translucent, turbid, and then finally dissolving back to an opaque solution at a critical temperature ranging from 44 to 70°C.

Kim and his colleagues then investigated the in situ gelation of a PEG-PLGA-PEG triblock copolymer that, being in an aqueous solution (33 wt.%), was injected subcutaneously into rats (57). Transparent gels formed immediately after subcutaneous injection, and these formed gels became strong enough to handle with forceps 24 h after injection. PEG-PLGA-PEG triblock copolymer hydrogels remained at subcutaneous sites 1 month after incubation with turbid appearance. This outcome is in sharp contrast to Pluronic hydrogels, which dissolve and disappear from the injection site within a few days. The relatively hydrophobic core of PEG-PLGA-PEG triblock copolymers prevents permeation of water molecules and, thus, results in a relatively stable core-shell micelle structure. These gels lost their mass approximately 30% after 1 month of incubation in the body, probably due to hydrolysis.

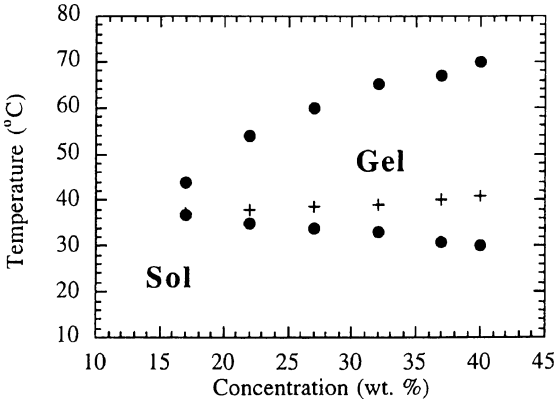


Figure 4 Phase diagram of PEG-PLGA-PEG (550-2810-550) triblock copolymer aqueous solution. Cross bar: temperature where the formed gels become turbid. (From Ref. 50.)

^{13}C NMR analyses revealed that degradation in the body caused PEG segments to be preferentially diffused out of the gels.

Kim and his colleagues (53) also investigated the drug release behavior from PEG-PLGA-PEG in situ formed gels. Two low molecular weight compounds, ketoprofen and spironolactone, were used as model drug molecules having different hydrophobicities. The relatively hydrophilic ketoprofen was released monotonously through diffusion mechanisms, with approximately 90% of the drug released within 5 days. In contrast, the more hydrophobic spironolactone was released and showed a sigmoid curve, with the release extending over 50 days. Given that the polymeric micellar structure was maintained within the triblock copolymer gels, the spironolactone molecules in PEG-rich shell layers were released mainly by a diffusion process, while drugs preferentially existing in the hydrophobic micelle core were released via diffusion and bulk micelle matrix degradation. Thus, the longer-term sustained release of drugs was achieved using PEG-PLGA-PEG triblock copolymer gels.

These triblock copolymer formulations are attractive as drug delivery depots because (i) their formulation requires no organic solvent; (ii) the triblock copolymer matrices can be stored as dry, solid forms before administration; (iii) drugs with a delivery vehicle can be injected directly by syringe so that no surgical operation is necessary; and (iv) the polymeric matrix is biodegradable, and the components come to have low molecular weight molecules so that those components are excreted by physiological mechanisms.

Zentner et al. (58) prepared PLGA-PEG-PLGA triblock copolymers via the bulk polymerization of PEG with lactide and glycolide in the presence of stannous 2-ethylhexanoate, for commercial delivery products, ReGel® (see structures in Fig. 3). These triblock copolymers have an analogous but inverted structure derived from the triblock systems reported by Kim and colleagues (51–55). Aqueous polymer solutions of more than 10 wt.% show three phases—solution, gel, and precipitate—depending on temperature, as shown in Figure 5. During the solution-to-gel transition, 23 wt.% ReGel showed

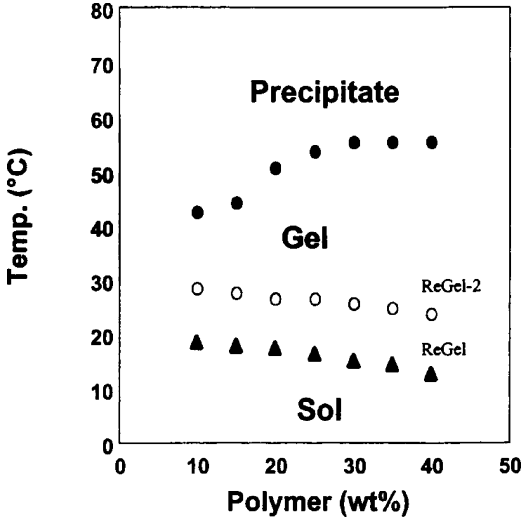


Figure 5 Phase diagram of ReGel®. (From Ref. 55.)

large viscosity changes (approximately four orders of magnitude) from 0.4 P in the sol state to 5700 P at the onset of gelation at 13.6°C. Such viscoelastic behavior is reproducible with repeated temperature changes. In vitro degradation of ReGel showed more rapid degradation at higher temperatures. Complete degradation of ReGel occurred after 6–8 weeks at 37°C, whereas, at low temperatures, polymers were stable for (for example) 20–30 weeks at 5°C and more than 2 years at –10°C. The in vivo degradation of ReGel after subcutaneous injection indicated that gel appearance dramatically changes between 2 and 4 weeks: initially gelled polymer matrices decrease in size during the first 2 weeks; then, they become a mixture of gel and a viscous liquid, and then a completely viscous liquid with no gel; finally, the matrices are completely absorbed into the body, and follow a simple hydrolysis mechanism.

Highly hydrophobic, and thus practically insoluble, drugs such as paclitaxel and cyclosporin A ($\sim$4 μg/mL), can be dissolved dramatically by mixing these drugs into a 23 wt.%

ReGel at 5°C, a solubility increase of 400 to >2000-fold. These drugs are stable in ReGel formulations, with more than 85% stability after 30 days of incubation at 37°C, and more than 99% after storage for one year at –10 and 5°C. Thus, drug stability is significantly improved by the use of ReGel formulations.

Zentner et al. (58) also evaluated in vitro paclitaxel release from the ReGel system and in comparison with the Pluronic F127 system. Drug molecules were completely released from the Pluronic F127 formulation within a 1-day period, indicating an unstable gel structure (Fig. 6). In sharp contrast, the paclitaxel release from ReGel systems showed a two-phase release pattern: a diffusion-governed mechanism for initial 14 days, and the combined mechanism of diffusion and polymer degradation for 50 days. Direct injection of paclitaxel containing ReGel solutions to solid tumor sites that had developed in mice resulted in the gelation of polymers only at injection sites, that is, within tumor tissues. Paclitaxel thus

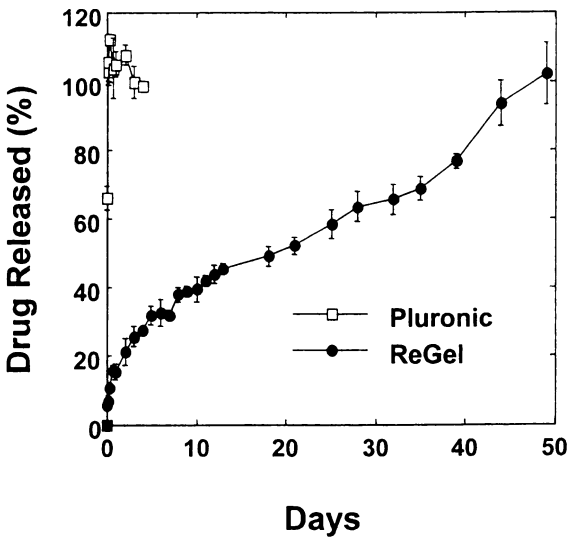


Figure 6 In vitro release of paclitaxel from ReGel® (23 w/w% in water) in comparison with that from Pluronic F-127. (From Ref. 55.)

affected not normal tissues but only tumor tissue. The drug remained at the tumor site 42 days after injection, by which time it had gradually decreased to 20%.

ReGel can also be utilized for the formulation of peptide drugs, like insulin (59), growth hormones (GH) and granulocyte colony-stimulating factor (G-CSF). In vitro protein drug release is monotonous and lasts for approximately 2 weeks after injection. Such differences may be due to the different molecular weights of drug molecules and the drug distribution within the gel matrix.

The degradation of the ReGel matrix results in the formation of lactic acid, glycolic acid, and PEG with a molecular weight of 1000 or 1450, which are all considered biocompatible. Thus, the ReGel formulation may be widely applicable to many types of drug molecules and peptides.

Jeong et al. (60) designed a relatively short-term drug delivery depot based on PEG grafted with biodegradable poly(lactide-co-glycolide) (PLGA) (see structure in Fig. 3). They initially reacted PEG (MW 1000) with glutaric anhydride to form carboxyl-terminated PEG, followed by chain extension with epoxy-PEG. During this reaction, pendant hydroxyl groups were also simultaneously introduced. These hydroxyl groups were then used to polymerize lactide and glycolide and, thus, to form hydrophobic, biodegradable grafted side chains. In water, PEG chains were hydrated and elongated, whereas PLGA chains were hydrophobic and condensed. These graft type polymers thus formed a polymer micelle structure 9 nm in diameter in water above their critical micelle concentration of 0.01–0.05 wt.%. Polymer solutions of more than 16 wt.% show a temperature-dependent sol-to-gel phase transition. These gels form two phases, water and precipitates, with an increase in temperature. Interestingly, polymer solutions at some concentrations remain in a gel phase at 37°C. Such properties allow for the use of the solutions as an injectable, in situ-gelling drug delivery depot. Aqueous polymer solutions of 22 wt.% show a lower viscosity of approximately 30 cP at 20°C, but induce gel formation when the temperature rises to 37°C. Gels remain unchanged for 7 days, after which point the gel matrix disintegrates,

resulting in a clear solution. These data indicate that in situ-formed PEG-g-PLGA gels can be used for drug delivery systems that require only short-term duration.

Chung et al. (61) prepared graft copolymers comprising a PLGA backbone with PEG-grafted chains by a one-pot ring-opening polymerization of lactide and glycolide in the presence of methoxy-PEG and epoxy-PEG (see structure in Fig. 3). These PLGA-g-PEG polymer solutions showed a temperature-dependent sol-to-gel phase transition at concentrations of 20–30 wt.% and a transition temperature of around 30°C. Gel formation occurred immediately after a temperature increase, with subcutaneous injection into rats resulting in the formation of a round-shaped gel at injection sites. PLGA-g-PEG gels remained at injection sites for more than 2 months. Thus, the long-term release of drugs can be achieved with these gels.

Jeong et al. (62) also used these two types of copolymers as a protein delivery depot or tissue engineering matrices. Two different formulations were prepared: (i) PEG-g-PLGA/PLGA-g-PEG (50/50 by weight) and (ii) PLGA-g-PEG alone. These graft copolymers contained three graft chains and had a number average molecular weight of ~6000. The two formulations were dissolved in phosphate buffer at 25 wt.% with insulin, and injected subcutaneously (35.54 mg/kg-rat) into streptozotocin-induced diabetic rats. After injection, blood glucose levels were monitored periodically: normal glucose levels were observed for 5 days after the injection of the PEG-g-PLGA/PLGA-g-PEG mixture, and 2 weeks in the case of PLGA-g-PEG alone. The former formulations, consisting of two different graft copolymers, were mechanically weaker than the latter, and faster erosion occurred in the body for mixtures of PEG-g-PLGA/PLGA-g-PEG. Tissues around gel implantation sites were analyzed histologically and, after one month, were found to exhibit minimal chronic inflammation. Thus, these in situ-formed gels can be used as delivery devices for peptides and proteins.

In situ formation of gels can also be applied so that target cells are fixed at desired sites and that the gel structures are maintained as long as the repaired tissues replace the gel

matrices. Such an approach, termed *cell delivery*, has recently received a great deal of attention from researchers. Results indicate that injected cells can, depending on the signal strength, respond to external signals and produce bioactive compounds (63–65).

Aoshima and colleagues (66–72) recently investigated the cationic living polymerization of a variety of vinyl ethers having oxyethylene side chains with ω-alkyl or hydroxyl groups in the presence of added bases. Owing to the characteristics of ionic polymerization, polymers synthesized in this fashion have a narrow molecular weight distribution. Thus, in these block copolymers, block lengths and polymer molecular weights are precisely regulated. The unique characteristics of these synthesized poly(vinyl ether)s are (i) phase separation temperatures controlled by changing side oxyethylene units or ω-alkyl groups, (ii) narrow molecular weight distribution resulting in a highly sensitive phase separation temperature, and (iii) block copolymers of two types of thermo-responsive poly(vinyl ether)s showing multiple phase separation temperatures, corresponding to that of each of the blocks. This is all in sharp contrast to the properties of random copoly(vinyl ether)s. While these polymers were being characterized, stimuli-responsive soluble-insoluble changes were observed. Further investigation revealed that the block copolymers formed micelles and physical gels similar to PEO-PPO-PEO triblock copolymers or PEO-*b*-poly(alkylene oxide)s. Figure 7 shows several types of poly(vinyl ether)s synthesized. They are classified as having (i) hydrophilic, (ii) thermo-responsive, and (iii) hydrophobic segments. Thermo-responsive physical gelation of polymer solutions can be obtained by the inclusion of hydrophilic segments and thermo-responsive segments, or of thermo-responsive segments and hydrophobic segments, in block copolymers.

Figure 8 (66) shows the typical viscosity changes of a poly[2-(2-ethoxy)ethoxyethyl vinyl ether]-b-poly(methoxyethyl vinyl ether) [$(EOEOVE)_{200}$-*b*-$MOVE_{400}$] solution upon temperature increase. Poly(EOEOVE) exhibits a phase separation temperature of 40°C, whereas polyMOVE shows a transition temperature of 70°C. In the block copolymer solution, the transparent solution forms a clear gel at 40°C,

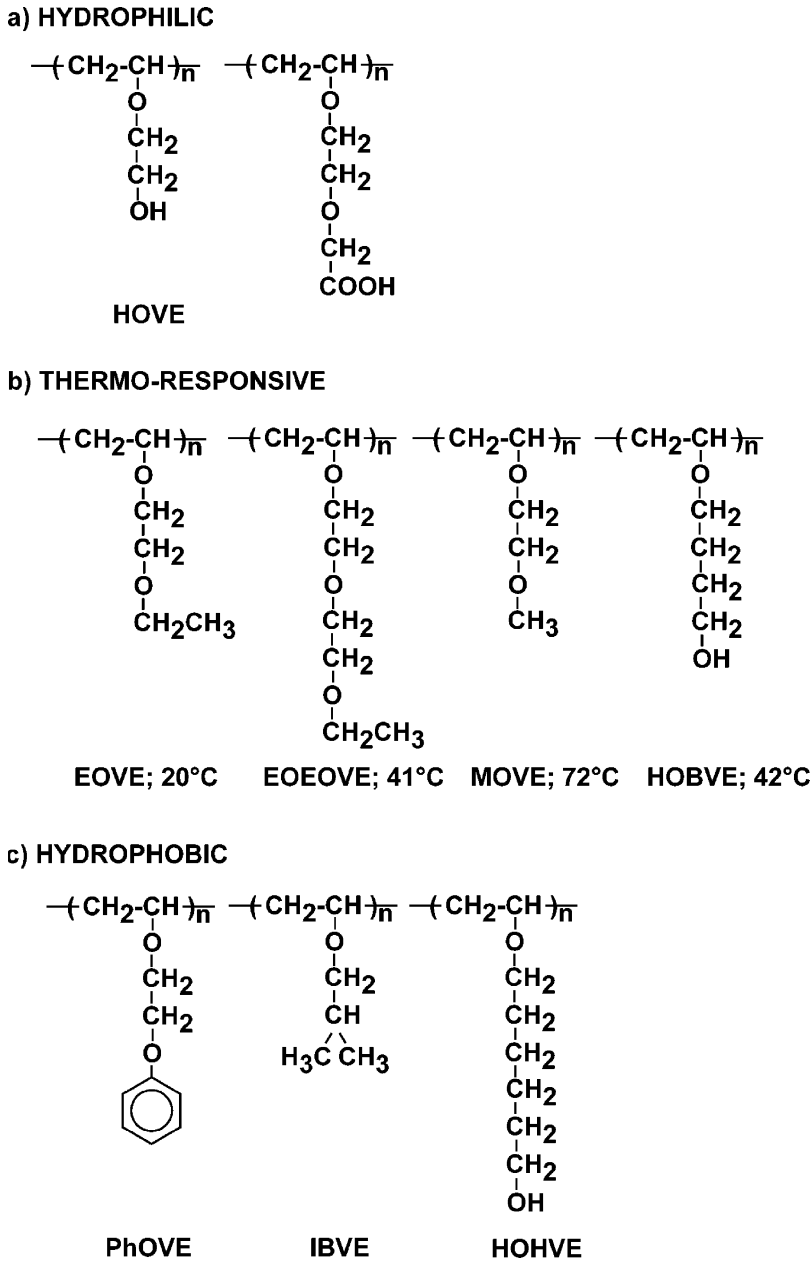


Figure 7 Structural formulae of functional poly(vinyl ether)s.

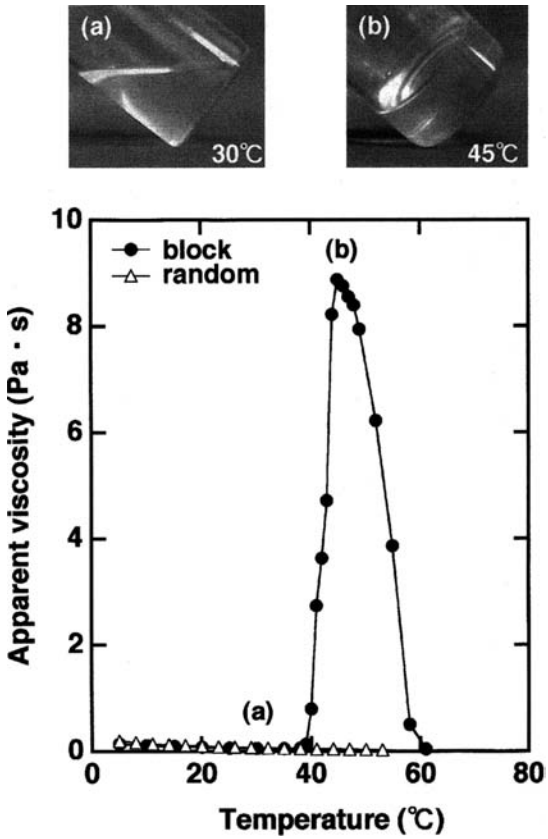


Figure 8 Temperature dependent apparent viscosity changes of 20 wt.% $EOEOVE_{200}$-*b*-$MOVE_{400}$. Solution appearances (a) at 30°C and (b) at 40°C are also indicated. (From Ref. 60.)

resulting in a sharp increase in solution viscosity. This gel phase is maintained between 40 and 55°C. A further temperature increase results in a hot clear liquid until 63°C is reached. Then, solution viscosity suddenly drops, and the solution becomes opaque to turbid above 63°C. In sharp contrast, random copolymers of EOEOVE and MOVE show not viscosity changes but precipitation at 53°C. Therefore, the structure of block copolymers is an important factor in temperature-responsive physical gelation.

To elucidate the physical gelation mechanisms of block poly(vinyl ether)s, Aoshima and colleagues utilized small-angle neutron scattering (SANS)(71) and differential scanning calorimetry (72) to study poly(ethoxyethyl vinyl ether)-*b*-poly(hydroxyethyl vinyl ether)(EOVE-HOVE). The block copolymers formed a micelle structure in aqueous solutions above the transition temperature of EOVE (20°C). At higher concentrations above 10 wt.%, micelles formed a crystal-like structure with a macrolattice formation, which should be the primary factor underlying physical gel formation at elevated temperatures. This unique property could be utilized to incorporate drug molecules from aqueous solutions and, thus, to formulate an injectable drug delivery system. Although such an application of these poly(vinyl ether)s and their block copolymers has not yet been reported to date, it could be beneficial to some researchers trying to develop new drug delivery devices because the appropriate selection of block components can result in the design of polymers that feature particular desired properties.

2.2. pH-Responsive Polymeric Hydrogel Systems

The gastrointestinal tract is known to possess a wide pH range, from a gastric pH of 1–2 to an intestinal tract pH of 7–8. Such significant changes could be utilized for the formation of pH-responsive drug delivery devices. Additionally, tumor sites and some sites of infection are known to have local acidic pH values amenable to pH-sensitive release methods.

Dong et al. (73) prepared pH- and thermally responsive gels for applications in protein delivery. They prepared macroporous hydrogels that consisted of *N*-isopropylacrylamide (IPAAm), acrylic acid, and divinyl-terminated polydimethylsiloxane in the presence of small amounts of the cross-linker methylenebisacrylamide. The resulting gels remained in an unswollen state at pH 1.4 and 37°C. However, at pH 7.4, gels swelled significantly owing to the dissociation of carboxyl groups of acrylic acids and formed carboxylate anions, which, in turn, formed large pores within the gel matrixes from the repulsion of the anionic groups. Protein

drugs were then released through the large pores formed by swelling. Incorporation of amylase as a model protein drug into these gels resulted in a high retention of enzymatic activity. Therefore, Dong and colleagues concluded that the thermo- and pH-sensitive macroporous hydrogels could be utilized for the gastrointestinal delivery of protein drugs.

Kang and Bae (74) prepared various types of sulfonamide-containing copolymers with *N*,*N*-dimethylacrylamide (DMAAm) as the main chain components. Because sulfonamide units have pH sensitivity, pH-dependent polymer solubility changes as well as the p*K*a shifts of precursor compounds and monomeric sulfonamide units after polymerization were studied. The introduction of methacryloyl groups and further polymerization induced an increase in the p*K*a values of the analogous units of sulfonamides. The pH-dependent solubility changes revealed that, with increasing sulfonamide units in the copolymers, the pH-dependent solubility transition increased from approximately pH 5 to pH 7, and at higher pH ranges, the copolymers were completely soluble in water. Interestingly, such solubility changes occurred within a narrow range of pH changes (ca. 0.2–0.3 pH units). Furthermore, the solubility transition was completely reversible without hysteresis. Such sulfonamide-containing polymers were used to form cross-linked hydrogels (75). The introduction of a methacryloyl group to sulfadimethoxine resulted in a polymerizable sulfonamide moiety. This sulfonamide monomer was then polymerized with DMAAm in the presence of the cross-linking agent, *N*,*N*′-methylenebisacrylamide so that hydrogels with different sulfonamide contents would form (Fig. 9). Kang and Bae then investigated the pH-responsive gel-swelling behavior. All the prepared gels swelled at higher pH regions, and as the sulfonamide content in the copolymer gels increased, the swelling ratio increased. A sharp swelling transition was observed, within a narrow pH range, for hydrogels that had higher sulfonamide contents (see Fig. 10). At lower pH ranges, hydrogen bonding between hydrogen atoms in sulfonamide groups and oxygen atoms of sulfonyl groups may stabilize weaker dispersive interactions from surrounding phenyl groups in sulfonamide units. Such

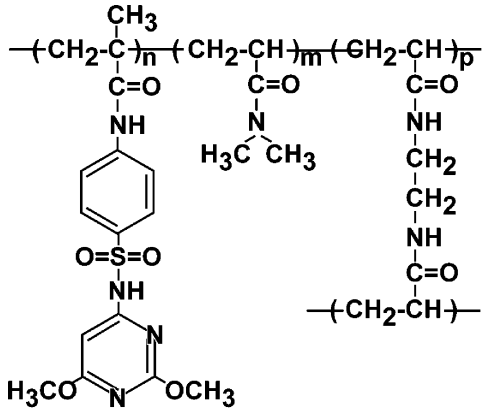


Figure 9 Structural formula of pH-responsive sulfonamide copolymer gels.

interactions induce the crystallization of sulfonamide units at low pH regions. This finding is confirmed by the x-ray diffraction measurements and apparent effective cross-linking density measurements of the sulfonamide gels at pH 6.8 and 7.4. Seeking possible applications in insulin delivery systems, the researchers further utilized pH-responsive sulfonamide-containing hydrogels for glucose-responsive

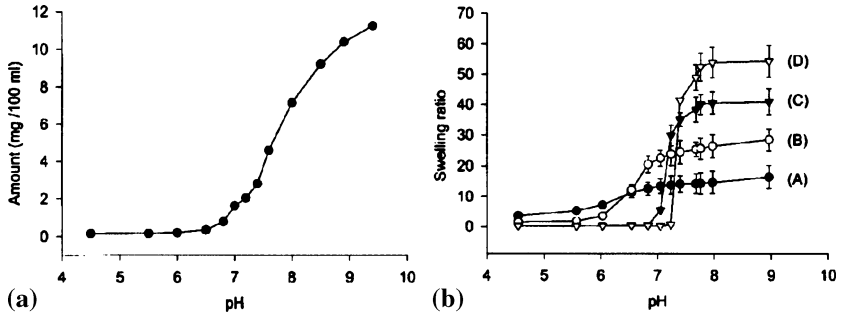


Figure 10 (a) pH-dependent solubility changes of sulfonamide monomer at 37°C. (b) pH-dependent equilibrium swelling ratio of the sulfonamide containing gels with (A) 10, (B) 20, (C) 30, (D) 40 mol%, respectively at 37°C. (From Ref. 69.)

swelling-deswelling control. They prepared sulfonamide-containing hydrogels in the presence of polymerizable glucose oxidase (GOx) and polymerizable catalase. GOx worked to form glucuronic acid from glucose and to maintain an acidic microenvironmental pH. Catalase stabilizes GOx by reducing the concentration of hydrogen peroxide, an outcome that has adverse effects on GOx activity. The prepared hydrogels showed pH-responsive gel-swelling changes, with linear swelling changes between pH 6.9 and 7.4. The researchers tested these gels for glucose-responsive swelling changes by immersing the gels in glucose solutions of different concentrations. With increasing glucose concentration, the microenvironment in the hydrogels became acidic owing to the formation of glucuronic acid within the gels, inducing gel shrinking through the neutralization of sulfonamide groups. By reducing the glucose concentration, re-swelling occurred. The glucose-responsive hydrogel swelling-deswelling changes were reproducible. The gels shrink with glucose concentration, a finding that warrants further investigation for applications in insulin delivery devices; moreover, the gel itself has the potential to improve the use of stimuli-responsive devices.

Traitel et al. (70) prepared poly(2-hydroxyethyl methacrylate-co-*N,N*-dimethylaminoethyl methacrylate) [poly(HEMA-co-DMAEMA)] hydrogels which entrapped GOx, catalase, and insulin. This hydrogel system proved to be the reverse of that presented by Kang and Bae (77). Glucose reacted with GOx to form glucuronic acid, which protonates hydrogel amino groups to induce gel-swelling. As the gel-swelling increased, the entrapped insulin was released. Furthermore, preliminary in vivo tests with rats revealed that released insulin shows the ability to decrease blood glucose levels. There was no observed tissue encapsulation around the hydrogels after implantation in the peritoneal cavity. Although confirmation remains necessary that no entrapped GOx and catalase leaked from the hydrogels, Gox-entrapped pH-responsive hydrogel systems show unique characteristics of insulin release.

Ma et al. (78) recently reported the pH-responsive micelle and physical gel formation of ABA-type triblock copolymers. They prepared ABA-type triblock copolymers

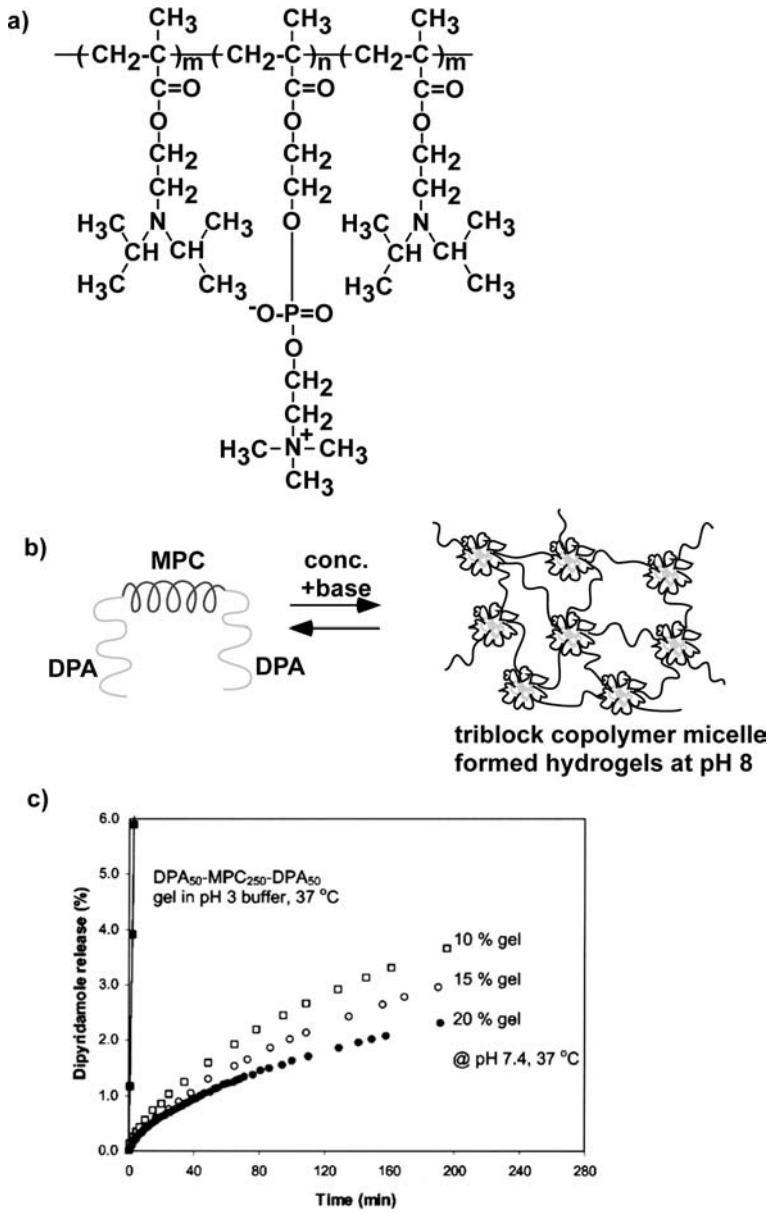


Figure 11 (*Caption on facing page*)

through an atom transfer radical polymerization (ATRP) technique to obtain polymers with narrower molecular weight distributions of 1.12–1.20. The A block consisted of either poly[2-(diisopropylamino) ethyl methacrylate] (DPA) or poly[2-(diethylamino)ethyl methacrylate] (DEA), and the B block contained poly([2-methacryloyloxyethyl phosphorylcholine]) (MPC). At low pH regions, amino groups in the A blocks were protonated and highly soluble in water, whereas these blocks deprotonated at neutral or higher pH ranges. The resulting triblock copolymers were soluble in water at acidic pH and became micelles of A blocks in ABA triblock copolymers to form hydrophobic aggregated cores, and the neutral hydrophilic B blocks formed the micelle corona (Figure 11a,b). At higher polymer concentrations in basic pH solution, physical gels are formed, as shown in Figure 11b. Thus, at physiological pH, drugs can be incorporated in the micelle cores, and a slow release of the drugs is achieved. Figure 11c shows the concentration-dependent release profiles of a model cardiovascular drug, dipyridamole, from DPA-MPC-DPA triblock copolymer gels. At pH 2, the polymer gels immediately dissolved and released drugs rapidly. Thus, pH-dependent controlled release can be achieved with the triblock copolymers indicated here.

2.3. Glucose-Responsive Hydrogel Systems for Possible Insulin-Release Devices

Insulin-dependent diabetes mellitus patients lack the pancreatic function that releases insulin in response to blood glucose levels. These patients require daily self-injections of

Figure 11 (*Facing page*) (a) Structural formula of pH-responsive triblock copolymer consisting of poly[(diisopropylamino)ethyl methacrylate]-*b*-poly(methacryloyloxyethyl phosphorylcholine)-*b*-poly[(diisopropylamino)ethyl methacrylate] prepared by ATRP technique. (b) Formation of macroscopic gels of concentrated solution of triblock copolymer. (c) Drug release behavior from triblock copolymer gels at 37°C and at pH 7.4. (From Ref. 72.)

an appropriate amount of insulin that help them to avoid hyperglycemia. Diabetic patients suffer from a gradual decline in the efficiency of various organs, leading to vision loss and long-term diseases. Severe conditions may even lead to patient death. Thus, injection of properly dosed insulin at proper times is required for insulin-dependent diabetes mellitus therapy. Self-injection of insulin, however, results in patient discomfort, varied bioavailability, and sometimes a hypoglycemic coma due to an overdose of insulin. Alternatively, insufficient insulin induces hyperglycemia and related complications. Therefore, the precise control of blood glucose levels with an effective, stimuli-responsive insulin release would be of great utility. Here, several formulations incorporating hydrogels for glucose concentration-dependent insulin release are reviewed.

Several insulin-release systems have already been introduced, and those systems utilize responsive gels and membranes with glucose (79,80). Ishihara et al. (81) prepared two types of gel membranes that respond to glucose and that regulate insulin permeability through the gel membranes. They prepared gel membranes which immobilized independently with GOx and nicotinamide. Glucose molecules were oxidized upon reaction with GOx, resulting in hydrogen peroxide oxidized gel-immobilized nicotinamide molecules to give positive charges in the gel membrane. These sequential reactions induced hydrophilic changes in the gel membranes and, thus, enhanced the membranes' permeability for the release of insulin. Kost et al. (82) and Albin et al. (83) prepared hydrogels consisting of hydroxyethyl methacrylate and *N,N*-dimethylaminoethyl methacrylate immobilized with GOx. Glucose molecules were converted, by means of the immobilized GOx, to glucuronic acid, and amino groups in the gels became protonated owing to the increased microenvironment acidity. Thus, the gels became swollen and induced insulin permeability that, at 400 mg/dl glucose, was 2.2–5.5 times higher than that at 0 mg/dl.

Obaidat and Park (84,85) prepared sol-gel transition polymers responsive to glucose. They prepared a water-soluble copolymer of acrylamide and allyl glucose. The resulting

polymers cross-linked in the presence of the sugar-recognizing lectin, concanavalin A (Con A), at side chain glucose moieties. Because binding constants of native glucose molecules are higher than those of glucose moieties on the copolymer side chains, an exchange reaction occurred between added glucose and copolymer glucose moieties, inducing a gel-to-solution phase transition. Such changes can be utilized for the permeation control of insulin. Thus, the authors used two-chamber cells separated with copolymer-Con A hydrogel membranes in order to investigate the glucose-dependent permeation control of insulin. Copolymer-Con A membranes showed glucose-dependent changes, though the response was slow, indicating that further optimization is needed to achieve sensitive membrane property alterations that can effectively facilitate the release of insulin in appropriate amounts and at appropriate times.

The above-mentioned examples all used proteins such as GOx and Con A. Exposure of these proteins and peptides to the body may cause an undesirable immune response upon contact. Therefore, these naturally derived proteins and peptides, and their whole systems, should be separated from the body using semi-permeable membranes. Utilization of totally synthetic polymer systems would be a versatile choice for the construction of new glucose-responsive insulin-release devices.

Kitano et al. (86–88) and Shiino et al. (89–92) prepared totally synthetic polymers with glucose-responsive functions. They focused on the unique characteristics of phenylboronic acid as a glucose-responsive moiety. Boronate is known to form reversible bondings with polyols such as cis diol sugar compounds like glucose (Fig. 12). The researchers prepared water-soluble copolymers containing phenylboronic acid side chains using m-acrylamidophenylboronic acid (AAPBA) and various water soluble monomers, including *N*-vinylpyrrolidone, acrylamide, and DMAAm. The resulting copolymers formed reversible complexes with poly(vinyl alcohol) as polyol compounds (86–88). These complexes dissociate with the addition of glucose in a concentration-dependent manner (Fig. 13) (88). Such complex formation and dissociation occurred owing to the different dissociation constants of phenylboronate anions with PVA or glucose. Utilizing the

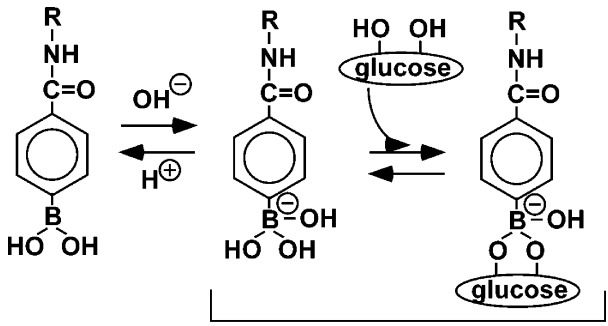


Figure 12 Schematic illustration of equilibria of alkylphenylboronic acid in aqueous solution in the presence of sugar.

polyol-binding characteristics of boronate anions, the researchers prepared polymeric microgel beads containing phenylboronic acid moieties (with diameters ranging from 100 to 400 μm) (90). At the same time, the researchers modified insulin with glucuronic acid to form a complex with boronate anions on the microgel beads. Insulin-bound microgel beads were then packed into mini-columns, and a glucose solution in phosphate-buffered saline was passed through the mini-column. Insulin was released as effluent in a glucose concentration-dependent manner. Insulin release from the mini-column can be modulated by the stepwise modulation of a glucose concentration from 80 to 200 mg/dl (anything above this range was called *hyperglycemic*). A superior characteristic of the mini-column systems is the recharge capability of gluconate insulin onto the phenylboronate anions in the column matrix beads. The system, however, had the disadvantage that glucose-responsive insulin release can be achieved only at pH 8.5, where boronate anions complex with gluconated insulin or blood glucose. Thus, it is necessary to reduce the working pH to physiological conditions, that is, to pH 7.4. To overcome this disadvantage, amino groups were incorporated proximal to phenylboronic acid moieties (91). The lone electron pair of the amino nitrogen atom coordinated

with the unoccupied orbital of the boron atom so as to allow the formation of stable complexes between polyol compounds and boronate anions, even at physiological pH 7.4. Shiino et al. (91) have prepared amine-containing phenylboronic acid microgel beads incorporating gluconated insulin. Amine-containing phenylboronic acid microgel beads were designed to release insulin in a glucose concentration-dependent manner at pH 7.4. If the daily insulin requirement in adult diabetes mellitus patients is 1 mg, only 1.36 cm^3 of insulin conjugated phenylboronic acid gel beads would be required to maintain normal blood glucose levels. The researchers compared their newly developed systems with the microcapsulated Con A-glucosylated insulin systems that Makino et al. (93) have created. In the case of microencapsulated Con A-glucosylated insulin, over 8000 cm^3 (8 liters!) of microcapsules were required to maintain similar therapeutic effects. This amount is too large for use in the human body. Thus, the amine-containing phenylboronic acid microgel beads systems not only display the realistic dosage capacity required for clinical applications, but also possess the feasible rechargeability of insulin once exhausted.

Kataoka et al. (94) developed dual stimuli-responsive hydrogels using 3-AAPBA as glucose-responsive moieties and IPAAm as thermo-responsive units (the prepared gels were termed *NB hydrogels*). The prepared gels with 10 mol % boronate units and in the absence of glucose in the medium showed a transition temperature at 22°C. The gels remained in a swollen state below the transition temperature of 22°C. The glucose addition to the medium induced an increase in the transition temperature to 36°C, that is, almost close to the physiological temperature, at 5 g/L glucose concentration, which is an extremely higher glucose concentration. Phenylboronic acid moieties were in an equilibrium state between the uncharged (undissociated) and charged anionic (dissociated) states. As the glucose concentration increased, the equilibrium shifted to produce more anionic charged boronate groups, inducing a hydrophilic and swollen gel (Fig. 12). Thus, the gel swelling-deswelling can be regulated with a glucose concentration at fixed temperatures. Insulin was then incorporated into dual

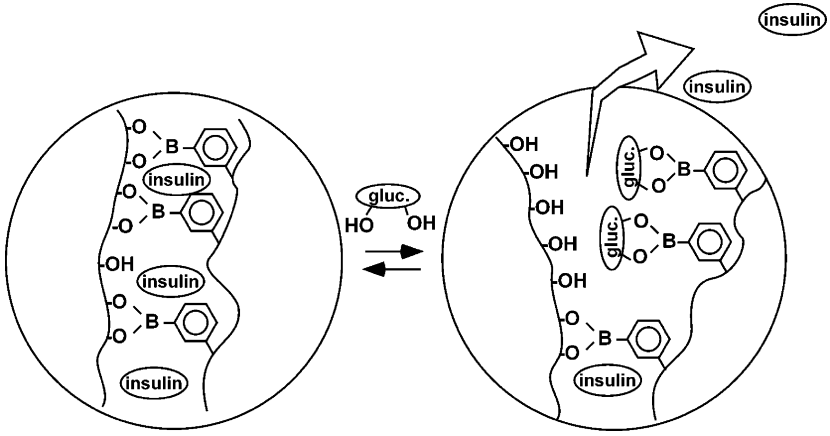


Figure 13 Schematic illustration of glucose-responsive insulin release from phenylboronic acid containing hydrogels cross-linked with poly(vinyl alcohol).

stimuli-responsive polymer gel beads and investigated for their glucose-responsive insulin-release behavior at pH 9.0 and at 28°C, where the gels showed significant and repetitive swelling-deswelling changes in glucose concentration. This finding is probably due to the bulk hydrophobicity of the polymer matrix, as well as the higher p*K*a value of phenylboronate anions. Insulin release was significantly suppressed below 10–20% at glucose concentrations ranging from 0 to 1.0 g/L during a 24-h incubation. More rapid insulin release was observed at glucose levels of 3.0 g/L, where approximately 80% of incorporated insulin was released during the initial 10 h. NB hydrogels were in a relatively hydrophobic and shrunken state below glucose concentrations of 1.0 g/L, and then swelled above this concentration owing to an increase in hydrophilicity arising from an increased number of boronate anions bound with glucose. Electrostatic charge was an additional driving force for hydrogel swelling. These results clearly indicate that a threshold in glucose concentration exists that alters hydrogel swelling-deswelling changes and thus the release of insulin from the swollen gels. The repetitive insulin-release characteristics were then investigated using thermo- and

glucose-responsive NB hydrogels. Figure 14 shows glucose-responsive insulin-release behavior from NB hydrogels at 28°C. These data show that insulin release is, without delay, synchronized with glucose-concentration changes. Interestingly, the gels stopped releasing insulin because decreases in glucose concentrations resulted in the formation of dense shrunken layers on the gel surfaces. The obtained gels were sensitive to glucose concentration changes, which affect gel-swelling states, although temperature ranges and operating pH must be optimized before clinical application can be achieved.

Matsumoto et al. (95) recently synthesized a new glucose-sensitive monomer, 4-(1,6-dioxo-2,5-diaza-7-oxamyl)phenylboronic acid (DDOPBA), possessing a low p*K*a value of ~7.8. They chose poly(*N*-isopropylmethacrylamide) (PNIPMAAm) because this polymer shows a phase transition temperature near the physiological temperature of 37°C. Then, they copolymerized PNIPMAAm with the newly developed monomer, DDOPBA (structural formulae shown in Fig. 15a). The researchers investigated the p*K*a values of the obtained copolymers by changing the pH, as well as the temperature of the solution. The apparent p*K*a value of DDOPBA is 7.79, which is approximately 0.4 units lower than that of previously used

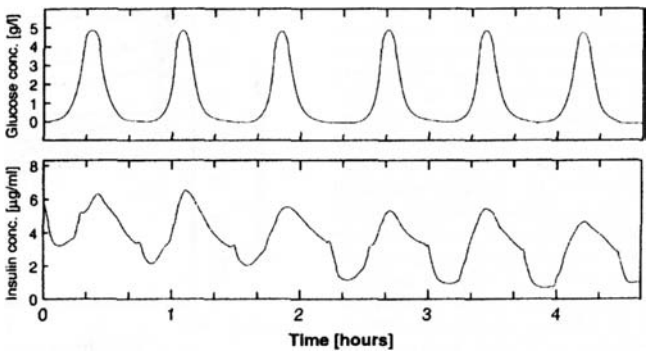


Figure 14 Glucose responsive insulin release from poly(*N*-isopropylacrylamide-co-acrylamidophenylboronic acid) copolymer gels. (From Ref. 88.)

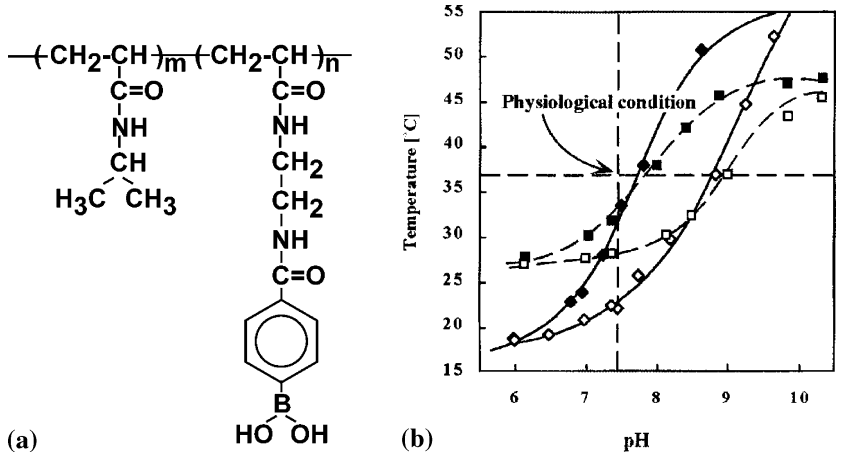


Figure 15 (a) Structural formula of poly[*N*-isopropylacrylamide-co-4-(1,6-dioxo-2,5-diaza-7-oxamyl]phenylboronic acid) [P(NIPAAm-DDOPBA)]. (b) Glucose and pH dependent changes in the LCST of 10% DDOPBA (square plot) and of 18.4% DDOPBA (diamond) in the presence (closed plots) and absence (open plots) of 5 g/L glucose. (From Ref. 89.)

(AAPBA) over a wide range of glucose concentrations. Thus, the researchers synthesized copolymers of NIPMAAm with 10 mol% of DDOPBA. Owing to the restricted main chain rotation by α-methyl groups on PNIPMAAm, the transition temperature increased relative to the PNIPAAm, although the hydrophobicity was higher in the PNIPMAAm than in the PNIPAAm. Figure 15b shows the pH- and glucose-responsive transition temperature changes of the newly developed copolymer of NIPMAAm and DDOPBA. The copolymer showed transition temperature changes that were similar to those observed in the control copolymer NIPAAm and the AAPBA, yet a distinctive difference was seen in the transition temperature ranges, shifting to higher regions to include the physiological temperatures range. Furthermore, it is noteworthy that the NIPMAAm-DDOPBA copolymer containing higher PBA moieties resulted in a decrease in the transition temperature; at the same time, however,

more drastic changes in the transition temperature were observed as pH increased. Moreover, a significant transition temperature change occurred in the presence of glucose near physiological pH 7.4. These results strongly suggest that the newly developed copolymers could be a promising material for the preparation of glucose-responsive insulin delivery devices operating at physiological temperature and pH ranges.

2.4. Other Stimuli-Responsive Hydrogel Systems

2.4.1. Electrically Responsive Hydrogel Systems

Several technologies are currently under investigation for the development of new drug delivery devices that can achieve chronotherapy, specifically, iontophoresis, infusion pumps, and sonophoresis (96).

Using polyelectrolyte gels as drug containers, Kishi et al. (97) prepared drug delivery devices responding to electrical stimuli to alter gel-swelling behavior. The gels responded to the on-off stimulus of electrical currents, which induced the gel swelling-shrinking that contributes to the release of drug molecules. The researchers prepared poly(sodium acrylate) microparticle gels containing pilocarpine as a model drug. During application of a direct electric current, the pilocarpine release increased in a current-dependent manner. However, the pilocarpine release did not stop upon termination of the electrical stimulus, since the prepared gels themselves maintained a highly swollen state. Thus, a complete on-off release regulation of drugs cannot be achieved with this polymer gel system.

Kwon et al. (98–100) prepared cross-linked poly(2-acrylamide-2-methylpropanesulfonic acid-co-butyl methacrylate) [P(AMPS-co-BMA)] hydrogels and evaluated the applicability of these hydrogels for electrical stimuli-responsive drug delivery devices. They used a cationic drug molecule, edrophonium chloride, within the negatively charged hydrogel. Rapid drug release from the hydrogels resulted from an application of electric fields through the ion exchange between positively charged drug molecules and protons at the cathode. The

squeezing effects arising from the electric field application induced rapid drug release from the gels, which increased as the voltages increased in a dose-dependent manner. Using the P(AMPS-co-BMA) hydrogels, an on-off drug release regulation was achieved under an on-off application of electric current.

Kwon et al. (101) further investigated the electric current-induced release of anionic heparin from a positively charged polyallylamine polyion complex. Rapid structural changes and an apparent dissociation of the polyion complex occurred upon application of an electric current. During the electric current application, the positively charged polyallylamine was neutralized at the cathode owing to the microenvironmental pH changes, and apparent dissociation of the polyion complex occurred. Although bioactive heparin was released by electric current application, polyallylamine was also released. Such positively charged molecules are incompatible in vivo, and these complications must be addressed before clinical applications can be considered.

2.4.2. Inflammation-Responsive Hydrogel Systems

Inflammatory reactions are commonly observed at injury sites. Inflammation-responsive cells like macrophages and polymorphonuclear cells play a key role in normal healing processes after injury. Hydroxyl radicals (•OH) are produced from the inflammation-responsive cells at the injured sites. Yui et al. (102,103) recently designed a hydroxyl radical-responsive drug delivery system that utilized the hydroxyl radicals produced at inflammation sites. The researchers utilized hyaluronic acid (HA), a linear mucopolysaccharide consisting of repeating units of *N*-acetyl-D-glucosamine and D-glucuronic acid. In the human body, HA is usually degraded by the specific enzyme hyaluronidase or by hydroxyl radicals. However, in a healthy state, the hyaluronidase-driven degradation of HA is unusual, and the majority of degradation occurs by hydroxyl radical exposure. For the preparation of inflammation-responsive hydrogels, HA was cross-linked with

ethylene glycol diglycidylether or polyglycerol polyglycidylether. A Fenton reaction was used to produce hydroxyl radicals, and the hydroxyl radical-induced degradation behavior of cross-linked HA hydrogels was observed. HA degradation in response to hydroxyl radicals was observed only at the surface of the gel, indicating that these gels exhibit surface erosion degradation. Further utilization of these hydrogels involved the introduction of microspheres as model drug carriers in the hydrogels, and the release behavior of microspheres from the gels was monitored. It was noted that the release of microspheres from this system also followed the surface erosion characteristics of the gels. In vivo degradation was also investigated. Surgery-induced inflammation and thus hydroxyl radical production at the surgery sites also degraded the HA hydrogels. Control HA gels did not degrade after long incubation times in the body. Thus, the prepared HA gels can be used in vivo for inflammation-induced drug delivery systems, such as incorporation with anti-inflammatory drugs and specifically for chronic inflammatory problems including rheumatoid arthritis.

2.4.3. Antigen-Responsive Hydrogel Systems

Recently Miyata et al. focused on naturally occurring bioactive proteins to develop new hydrogels having antigens and entrapped antibodies within the gel matrices (104–106). The obtained poly(acrylamide) (PAAm) hydrogels had chemically cross-linked points of MBAAm; moreover, the vinyl-conjugated antigen and antibody complex formation through non-covalent multiple bonds served to further cross-link the gel. When free rabbit IgG antigen molecules were added to the solution with immersed hydrogels, an exchange reaction occurred between the antigen molecules bound to the hydrogels' main chains and the added free antigen owing to the difference in the binding constants to the goat anti-rabbit IgG antibodies. This antigen competitive exchange resulted in a decreased number of cross-linking points in the hydrogels, and thus promoted the swelling of hydrogels (Fig. 16). Such changes are very antigen specific, so that the addition of other

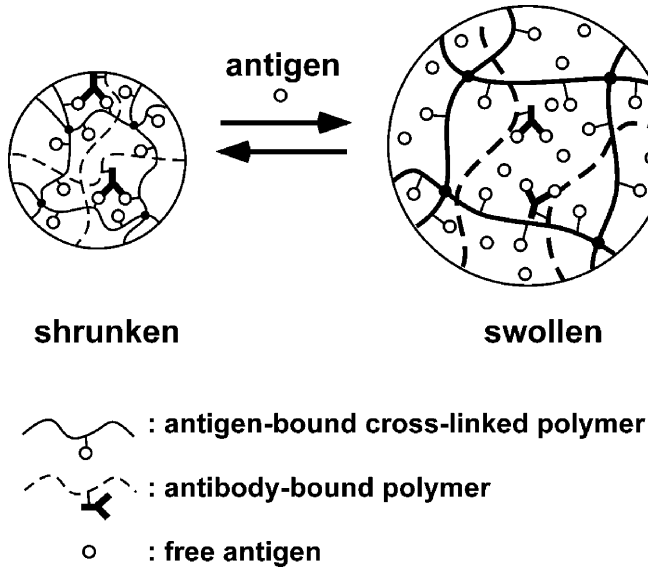


Figure 16 Swelling-deswelling changes of antigen-responsive hydrogels.

antigens including goat IgG did not alter hydrogel swelling, since there is no specific binding between goat IgG and goat anti-rabbit IgG and, thus, no decrease in gel cross-linking points. However, hydrogel shrinking did not occur because the newly formed antigen-antibody complexes were no longer mutually connected within the gel matrices. Thus, Miyata et al. then prepared semi-interpenetrating polymer network (semi-IPN) gels of antigen-conjugated cross-linked hydrogels synthesized in the presence of a polymerized antibody. The polymerized antibody does not easily diffuse out of these semi-IPNs, since the polymer chains are entangled with three-dimensionally cross-linked antigen polymer networks. Through the semi-IPN gels, antigen concentration-dependent repetitive gel swelling-deswelling kinetics was obtained. Antigen addition induced the dissociation of the polymerized antigen-antibody complex within the semi-IPN hydrogels, and the gels swelled. On the contrary, the swollen gels began to shrink gradually in the absence of free antigen molecules.

Antigen containing polymer chains were entangled within the cross-linked gels, and thus the micro-environmental antigen concentration increased when in the presence of polymerized antigens and in the absence of free antigens. Therefore, gel deswelling occurred repeatedly. Using two-chamber cells separated with antigen-antibody semi-IPN hydrogels, the researchers then investigated the antibody concentration-dependent permeation control of hemoglobin. Stepwise antigen exposure of the antigen-antibody complex-forming semi-IPN showed repeating swelling-deswelling changes, and on-off permeation control of hemoglobin was synchronized through the semi-IPN gel membranes. The prepared semi-IPN hydrogel systems can be used as possible drug delivery systems for the antigen-dependent release of drug molecules.

3. CONCLUSIONS

In the present chapter, stimuli-responsive hydrogels and their applications in stimuli-responsive drug delivery systems were reviewed. A variety of stimuli can be exploited for the design of new drug delivery devices that can be used to satisfy a variety of options for controlled release. In all cases, effective gel systems should include (i) sensor functions specific to the desired stimulus, (ii) signal processing with which to alter appropriate gel physicochemical properties and subsequent drug release profiles, (iii) the release of an adequate amount of a drug (dose) over an appropriate time frame, and (iv) a reliable halt to drug release after the stimulus signal returns to normal. Synthetic polymers possess attractive potential because experts in the field can easily alter their design in order to achieve the desired characteristics of hydrogels that respond to many stimuli of physiological and technical relevance. A more precise design of new materials and innovative gel architectures will facilitate novel fine-tuned drug delivery devices that, by responding to desired stimuli, offer the medical community more effective and reliable treatments of a variety of diseases.

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