

Stimuli-sensitive hydrogels: ideal carriers for chronobiology and chronotherapy

NICHOLAS A. PEPPAS* and WILLIAM LEOBANDUNG†

*The University of Texas, Departments of Chemical Engineering and Biomedical Engineering and
Division of Pharmaceutics, 1 University Station, C0400, CPE 3.466, Austin, TX 78712-0231, USA*

Received 21 August 2003; accepted 8 December 2003

Abstract—The development of solid-phase peptide synthesis in the early 1960s and recombinant DNA technology in the early 1970s boosted the scientific interest of utilizing proteins and peptides as potential therapeutic agents to battle poorly controlled diseases. While there has been rapid progress in the development and synthesis of new proteins and peptides as potential therapeutic agents, the formulation and development of the associated delivery systems is lacking. The development of delivery systems is equally important due to the problems of stability, low bioavailability and short half-life of proteins and peptides. The main problem in this field is that low stability leads to low bioavailability. In this review we draw attention to chrono-pharmacological drug-delivery systems, which can be used to match the delivery of therapeutic agents with the biological rhythm. They are very important especially in endocrinology and in vaccine therapy. We show that the treatment of hypopituitary dwarfism by administration of human growth-hormone-releasing hormone (GHRH) is more effective when GHRH is administered in a pulsatile manner that exhibits a period characteristic of the patient's circadian rhythm. Here we examine how to design novel chrono-pharmacological drug-delivery systems that should be able to release the therapeutic agents at predetermined intervals.

Key words: Chronobiology; chronotherapy; stimuli-responsive polymer; hydrogels; protein delivery.

INTRODUCTION

Chronobiology and chronotherapy

The need of carriers that exhibit oscillatory behavior of the releasing drugs, peptides or proteins has been a significant problem of drug design and formulation in recent years. The fields of pharmaceutics and biomedical engineering have been concerned with the choice of medication and the level of dosage of such drugs. It is a common

*To whom correspondence should be addressed. Tel.: (1-512) 471-6644. Fax: (1-512) 471-8227.
E-mail: peppas@che.utexas.edu

†Present address: Intel, 5200 Northeast Elam Young Parkway, Hillsboro, OR 97124, USA.

practice in clinical pharmacology to consider pharmacokinetic parameters that would allow release unaffected by the time of day of drug administration. However, over the past four decades, new findings have shown that the time of treatment is also an important issue in drug delivery. Researchers have concluded that the chronological point of treatment may affect the outcome of a host of conditions including hypertension, asthma, ulcers, arthritis, cholesterol and even cancer.

Table 1.

Drugs for which daily variations in their effects were reported in clinical studies (adopted from Ref. [7])

Cardiovascular active drugs

β -Blockers

Oxprenolol

Propranolol

Timolol

β -Agonists

Midodrine

Terbutaline

Adrenaline

Calcium channel blockers

Nifedipine

Diltiazem

Diuretics

Indapamide

Xipamide

Piretanide

Torsemide

Organic nitrates

Isosorbide-dinitrate

Isosorbide-5-mononitrate

Anticancer drugs

cis-Platin

Doxorubicin

Bleomycin

Methotrexate

Antiasthmatic drugs

Theophylline

Budesonide

Psychotropic drugs

Diazepam

Haloperidol

Phenylpropanolamine

H₁-antihistamine

Clemastine

Terfenadine

Cyproheptadine

Mequitazin

There is convincing evidence that constant drug delivery is not always effective from a pharmacological point of view. It is well established that nearly all functions of the body, including those influencing pharmacokinetic parameters, display significant daily variations or patterns.

Recently, yet another paradigm has appeared! It has been generally assumed that constant drug infusion leads to a constant drug concentration, which in turn leads to constant drug effects. However, a constant infusion of ranitidine (an H₂ blocker prescribed for gastric ulcers) over a period of 24 h did not lead to a constant effect. The increase in gastric pH caused by ranitidine was lower during the nightly hours of drug infusion than during daytime, indicating that there might be a partial nocturnal resistance to H₂-receptor blockade [1, 2]. Numerous other drugs show daily variations of their effect in clinical studies, as summarized in Table 1.

These new findings have led to the development of a new research area, chronopharmacology, i.e. the study of coordinating biological rhythms (chronobiology) with medical treatment. Chronobiology is the study of the temporal relationships of biological phenomena. Adaptability to the influence of the circadian patterns is apparent in that all organisms incorporate and retain this vital circadian periodicity in their genetic code.

The genes that control these circadian rhythms have been identified and isolated, for example in mouse, golden hamster and neurospora [3–7]. Konopka and Benzer [3] were able to identify a region on the X chromosome of *Drosophila melanogaster* that controlled the period in the eclosion rhythm of three mutants. Bargiello *et al.* [4] showed that a fragment of the *per* gene injected into embryos of an arrhythmic mutant of *Drosophila melanogaster* could restore rhythmicity in eclosion. Recently, the precise feedback regulation of the clock gene in the fruit fly has been unraveled [5–7].

Chronopharmacology does not involve new medicines but uses old ones in a different approach. Chronopharmacology takes a person's circadian rhythm into consideration in determining the timing and sometimes the quantity of medication to optimize a drug's desired effects and minimize undesirable side-effects. Revising the dosing schedule, reformulating a drug so its release into blood stream is delayed, or using programmable pumps that deliver medicine at precise intervals are some of the simple improvements that may harvest enormous benefits.

Many chronic and acute disorders have a prominent circadian pattern of symptom appearance and severity as summarized in Table 2. For example,

- (i) allergic rhinitis is an early morning disorder;
- (ii) patients with epilepsy have seizures during the day only, or the night only, although some may have a more random pattern;
- (iii) a H₂-blocker is more effective in treating peptic ulcers when the acid secretion of the stomach is the highest (during late afternoon) [1];
- (iv) asthma attacks usually occur during the night [8–10];

- (v) thrombotic stroke risk is greatest in the morning while hemorrhagic stroke it is in the late evening; and
- (vi) cholesterol medications are more effective if prescribed with the supper meal because clinical trials have shown that more cholesterol and other harmful lipids are synthesized in the late afternoon and evening hours [11].

There are other cardiovascular diseases that show this circadian rhythm, such as myocardial infarction, stroke, high blood pressure and angina pectoris [12–15].

Promising research is currently carried out in the area of cancer chronotherapeutics [16–21]. Doxorubicin, *cis*-platin and their analogs, 5-fluorouracil and 5-fluoro-2'-deoxyuridine, have been studied in the context of their circadian pharmacodynamics and toxicology [22, 23]. The outcome of these studies clearly shows that proper timing of their administration reduces drug toxicity and allows for substantial increase in the tolerated dose, which in turn results in better treatment efficacy and greater comfort for patients. The reasoning behind the increase of the tolerated dose is that a normal cell divides every 24 h, while a cancerous cell loses its biological timing and starts multiplying at a very high rate. If chemotherapeutic agents can be infused at the right time when the normal cell is not dividing, then the chemotherapeutic agents will have less effect on the normal cell.

The chronotherapeutical approach, though possibly expensive at the beginning, can be cost effective in the long run. If we can deliver the right drug at the right time and, in so doing, can minimize medical crises and side effects, we can eventually lower the cost and improve compliance. For example, if we can prevent end-organ damage to the heart, kidneys and eyes, which can result from hypertension, there will be great cost saving in the long term. While it seems natural and logical to simply change dosing schedules according to chronobiology, not all medications lend themselves to this approach. A drug that is suitable for chronotherapy has to have the right biological characteristics.

Table 2.

Circadian rhythm of many clinical diseases that have been studied*

Disease or syndrome	Circadian rhythmicity
Allergic rhinitis	Symptoms worse in early morning
Bronchial asthma	Exacerbations more common during the sleep period
Arthritis, rheumatoid	Symptoms are most intense upon awakening
Osteoarthritis	Symptoms worse in the middle/latter portion of the day
Angina pectoris	Chest pain and ECG changes more common during the early morning
Myocardial infarction	Incidence greatest in the early morning
Peptic ulcers disease	Symptoms worse after gastric emptying and in the early morning (sleep period)
Stroke	Incidence greatest in early morning

* From communications with M. Smolensky (University of Texas, Health Sciences, Houston, TX, USA).

Chronotherapy in growth hormone deficiency

One application of chronopharmacological systems that is of utmost interest is the treatment of growth hormone deficiency. Human growth hormone (hGH) is a protein of 191 amino acids with a molecular mass of 22 kDa. It is responsible for a wide range of growth-promoting effects in the body. It has a unique role in promoting longitudinal bone growth and an important function in the regulation of protein, lipid and carbohydrate metabolism. GH is secreted from the anterior pituitary gland in a pulsatile pattern. The pulsatility is controlled by the coordinated release of stimulatory (growth-hormone-releasing hormone; GHRH) and inhibitory (somatostatin; SRIF) hypothalamic peptides [24, 25].

Figure 1 shows the tight and complex regulation of secretion of GH by the hypothalamus. The GHRH secreted by the hypothalamus stimulates the pituitary gland to secrete GH. GH goes on to stimulate the release of insulin-like growth factor-1 (IGF-1) and stimulates many metabolic effects, as mentioned above. GH together with IGF-1 also stimulate the release of SRIF by the hypothalamus, which further inhibits the release of GH and GHRH.

Once the release of GH is stopped, the release of SRIF is also stopped because there is no more stimulant for the hypothalamus. Exclusion of SRIF results in release of GHRH again. Thus, the close loop system starts again resulting in a circadian pattern of GH in the body. This complex control results in a pulsatile GH profile, with a huge serum GH peak several hours after sleep onset, with several distinctive peaks afterwards.

The importance of GH in our body is unquestionable. Lack of GH causes short stature and abnormality in the metabolism of fat, lipid and carbohydrates. This abnormality in metabolism causes the build up of fat in people suffering from GH deficiency.

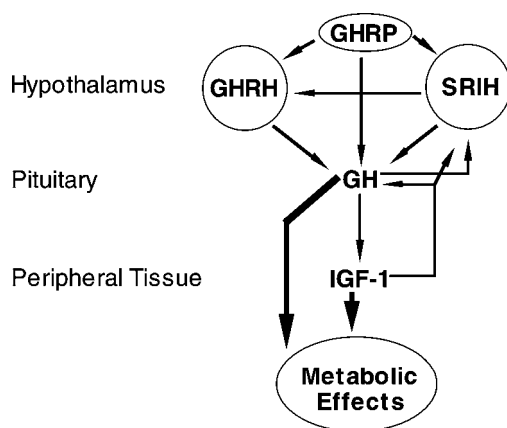


Figure 1. The tight regulation that controls the release of growth hormone by the anterior pituitary gland controlled by the growth hormone releasing hormone and somatostatin by the hypothalamus.

The causes behind the GH deficiency have been classified into three major groups:

- (i) genetically determined;
- (ii) lesions or injuries involving the hypothalamic or pituitary axis;
- (iii) idiopathic.

In the case of genetically determined deficiency, the GH deficiency is caused by the lack of the genetic code to produce the GH. In the second case, due to injuries related to the hypothalamic or pituitary axis, the body cannot produce GH or GHRH and SRIH. The first two types of GH deficiency can be treated by supplying GH exogenously. The most common deficiency is the idiopathic type. This type of GH insufficiency is due to the inability of the body to stimulate the release of GH from the pituitary gland because of the damage to the hypothalamus. Hence, the body is unable to produce GHRH to stimulate the release of GH. This type of GH insufficiency can be treated by either supplying GHRH or GH exogenously.

The frequency of idiopathic growth hormone deficiencies in newborn children ranges from 1 in 4000 to 1 in 10 000 births [26–28]. In children, low plasma levels of GH are responsible for short stature. Meanwhile in adults, age-related reductions in circulating GH cause a decrease in lean body mass, an increase in body fat and a decrease in bone mineral density [29–31].

The undesirable effects of low circulating levels of GH can be diminished by the administration of hGH, which is available through recombinant DNA technology. However, injection of this hormone does not mimic the normal pulsatile secretory pattern [24]. While this pharmacological dosing regimen may correct many of the effects of low circulating levels of GH, a more physiological pattern of administration could be safer and perhaps more effective [24, 32]. Several concerns about the safety and efficacy of pharmacological doses of the hormone in adults have been raised, including hyperglycemia, hypertension and carpal tunnel syndrome [33–37].

Following the proposition by Reichlin [38, 39] of the existence of a GHRH and the substantiation by Deuben and Meites [40] of its presence in hypothalamic extracts, numerous research groups had sought to characterize this elusive hormone. In 1982, peptides with growth hormone releasing activity were isolated from human pancreatic islet tumors obtained from patients with acromegaly, and were characterized independently by Rivier *et al.* [41] and Guillemin *et al.* [42]. The isolation and identification of GHRH has been followed by an astonishing rate of knowledge accumulation. Within a period of slightly more than 3 years since the structure of the GHRH was determined, nearly 500 research papers were published regarding this hormone [43]. Extensive knowledge of its chemistry, molecular biology, anatomy, physiology and pathology has been gathered and clinical trials have proceeded faster than with any other of the hypophysiotropic hormones. New insight has been gained with respect to the pathogenesis of both GH deficiency and GH excess states, and the use of GHRH and its analogs as diagnostic and therapeutic agents has been established.

GHRH, a 44-amino-acid peptide with a molecular mass of 5 kDa, can be administered to increase the systemic GH level. Endogenously, GHRH is synthesized in specialized neurons within the arcuate nucleus of the hypothalamus and released at the level of the median eminence into the pituitary portal vasculature. GHRH travels to the anterior pituitary gland where it binds with high affinity to a member of the G-protein-coupled receptor gene family [44]. Following activation of the GHRH receptor, adenylate cyclase is stimulated which results in an increase in intracellular levels of cAMP and, as a result, GH gene expression is increased [45]. In addition, receptor activation stimulates a voltage-dependent stimulus secretion coupled event mediated by an influx in extracellular calcium into the cell [46, 47]. Thus, GHRH receptor activation not only stimulates an acute release of GH from these cells, but also synchronizes spontaneous co-activation of neighboring cells. This phenomenon is thought to increase pituitary response to subsequent GHRH secretion as well as to organize and to amplify autonomous GH secretion [48]. Hence, administration of GHRH is a preferred method for systemic GH increase. However, one needs to understand that not all of the growth hormone deficiency can be treated with GHRH, only the idiopathic type as explained above.

Chronotherapy in diabetes

Another application of a chronopharmacological system is in the area of insulin-dependent diabetes mellitus (IDDM). Although the main physiological stimulus for insulin secretion in humans is food intake, the extent of the insulin response after food intake may also depend on the time of day. The circadian pattern of glucose metabolism and insulin secretion has been reported [49–57]. Current treatment of IDDM includes the injection of insulin subcutaneously after each meal, typically three times a day. This treatment with multiple injections creates patient discomfort and results in lower patient compliance. A system implanted under the skin, which would provide multiple discharges of insulin per day ‘matching each meal period’ could eliminate or moderate the multiple injection with multiple injections treatment.

STIMULI-RESPONSIVE HYDROGELS IN CHRONOTHERAPEUTIC SYSTEMS

The aim of the remaining portion of this review is to show how researchers have addressed the question of creating new chronopharmacological drug delivery systems for protein administration. Since proteins are sensitive molecules, we need to devise systems that can protect them. Hydrogels are a natural choice as chronotherapeutic carriers due to their unique physicochemical and biological properties.

The use of environmentally-sensitive hydrogels as carriers that can provide conditions of chronotherapeutic behavior is based on the premise that externally triggered events associated with the circadian rhythm will cause the release of an active agent at predetermined intervals. These triggering effects may be:

- (i) time-dependent and chronically-specific changes in pH, ionic strength or other physiological parameters that can lead to specific drug release;
- (ii) structural changes of a hydrogel carrier (mostly in the form of swelling/syneresis cycles) that are triggered by molecular recognition of specific biologicals secreted as a result of a specific chronopharmacological action; or
- (iii) phase erosion (by dissolution or biodegradation) of carriers due to significant external physiological changes.

As it is well known, hydrogels are three-dimensional structures that can imbibe a large amount of water. They are mainly composed of swellable hydrophilic polymers due to the presence of chemical or physical crosslinks. The physical crosslinks can be crystalline regions, inter-polymer entanglements or weak interactions such as van der Waals forces or hydrogen bonds.

Factors affecting hydrogel swelling

The development of chronotherapeutic systems is based on careful design of these hydrogel carriers to achieve a specific release pattern. For example, for a desired delivery system that would release two doses of a drug on specific intervals in the morning, a hydrogel carrier can be designed, based on slowly 'dissolving' crystalline junctions that will 'melt out' and release their contents in eight or then hours after administration, assuming drug administration before the patient goes to sleep.

Therefore, numerous physiochemical or structural parameters of the hydrogel can be used or manipulated for this release. For purposes of generalization, we will concentrate now on pulsatile or oscillatory delivery of such drugs, although we recognize that these examples will not affect the general nature of the problem.

For example, the degree of crosslinking, as controlled by the co-monomer crosslinking ratio and solvent content during preparation, is an important factor that affects the swelling of hydrogels. Highly crosslinked hydrogels have a tighter structure and swell less compared to loosely crosslinked hydrogels.

The chemical structure of the polymer also affects the swelling ratio of the hydrogels. Hydrogels containing hydrophilic groups swell to a higher degree compared to the ones containing hydrophobic groups. Hydrophobic groups aggregate leading to collapse of the hydrogel structure in the presence of water, thus minimizing their exposure to water molecules. Hydrophobic 'collapse at will' is one of several mechanisms of drug delivery at specified intervals by a 'squeezing' release process.

Swelling of stimuli-sensitive hydrogels can be affected by the change of the temperature, ionic strength and pH of the swelling medium. While stimuli-sensitive hydrogels are characterized by their equilibrium or quasi-equilibrium swelling ratios, chronotherapeutic systems are usually oral formulations taken in dry form. Therefore, their dynamic swelling kinetics is of utmost importance. The swelling kinetics of hydrogels can be diffusion-controlled (Fickian) and relaxation-controlled (Case II) swelling. When water diffusion into the hydrogel occurs much faster than

the relaxation of the polymer chains, the swelling kinetics is diffusion-controlled, or the solvent transport is controlled by a concentration gradient. For glassy polymer, the rate of polymer relaxation is the rate-limiting step to penetrant transport and, hence, a Case-II transport is observed. A nice mathematical analysis of the dynamics of swelling is presented by Peppas and Colombo [59].

Assurance of the integrity of drug-delivery devices during their lifetime of application is very important in order to obtain FDA approval, unless the device is designed as a biodegradable system. Drug-delivery systems designed to protect sensitive therapeutic agents, such as proteins, must maintain their integrity in order to protect proteins until they are released from the systems.

Modification of the degree of crystallinity, complexation or crosslinking has been utilized to achieve desired hydrogel stability. Therefore, hydrogel carriers can be designed that consist of layers of progressively increasing degrees of crystallinity or complexation from the surface to the center of the device. Thus, an incorporated drug will be released faster from the surface and then more slowly from the center. With appropriate compartmentalization, this hydrogel structure can lead to distinct 'spikes' of drug release, as we will show later in this review.

Stimuli-sensitive hydrogels

Stimuli-sensitive hydrogels have the ability to respond to changes of their external environment, thus becoming prime candidates for chronobiological applications. They can exhibit dramatic changes in their swelling behavior, network structure, permeability, or stability in response to changes in the pH or ionic strength of the surrounding fluid or temperature [60]. Other hydrogels have the ability to respond to applied electrical or magnetic fields. Yet, other hydrogels may respond to recognition mechanisms of specific biologicals, e.g. to changes in concentrations of glucose. Because of this unique property, such materials can be used in a wide variety of applications such as separation membranes, biosensors, artificial muscles, chemical valves and drug advanced chronobiological delivery devices.

pH-sensitive hydrogels. Ionic hydrogels are swollen polymer networks containing pendent groups, such as carboxylic or sulfonic acid, which show sudden or gradual change in their dynamic and equilibrium swelling behavior as a result of a change in the external pH. Ionization occurs when the pH of the environment is above the pK_a of the ionizable group [61–75]. As ionization increases (increased system pH), the number of fixed charges increases resulting in increased electrostatic repulsions between the chains. This, in turn, results (i) in an increased hydrophilicity of the network and (ii) in electrostatic repulsion, thus resulting in greater swelling ratio.

On the other side, cationic materials contain pendent groups such as amines [60–72, 76–78]. These groups ionize in media, which are at a pH below the pK_b of the ionizable species. Thus, in a low-pH environment, ionization increases causing increased electrostatic repulsions. The hydrogels become increasingly hydrophilic

and swell to a highly swelling ratio. More importantly, the associated drug-diffusion coefficient has been shown to increase up to three orders of magnitude.

The pH-sensitivity characteristics of these gels can be exploited for applications in a wide variety of biomedical applications such as mucoadhesive controlled release devices, prodrugs and adjuvants, and biocompatible materials [58]. The swelling of polyelectrolyte gels is significantly affected by the ionic strength of the swelling agent [61–79]. As the ionic strength of the swelling agent increases, the concentration of ions within the gel must increase in order to satisfy the Donnan equilibrium. The swelling force is reduced due to increased gel-counterion interaction and a decrease in the osmotic swelling forces.

Numerous researchers have studied the dynamic swelling of pH-sensitive networks. Katchalsky and associates [61, 63, 64] established that the collapse and expansion of poly(methacrylic acid) gels occurred reversibly by simply adjusting the pH of the fluid. Ohmine and Tanaka [74] observed the sudden collapse of ionic networks in response to sudden changes in the ionic strength of the swelling medium. Studies by Khare and Peppas [75] examined the swelling kinetics of poly(methacrylic acid) or poly(acrylic acid) with poly(hydroxyethyl methacrylate). They observed pH- and ionic strength-dependent swelling kinetics in these gels.

Temperature-sensitive hydrogels. Temperature-sensitive hydrogels exhibit swelling ratios that change in response to a change in temperature of the environment. Generally, the temperature-sensitive hydrogels can be divided into positive and negative thermosensitive hydrogels. Hydrogels with positive thermosensitivity contain mostly hydrophilic monomers and experience increased swelling with increasing temperature. Those with negative thermosensitivity are composed of monomers such as N-methylacrylamide, N,N-dimethylacrylamide and N-isopropylacrylamide, which contain hydrophobic substituents; they exhibit increased swelling with decreasing temperature [80].

Typical temperature-sensitive hydrogels [81] are prepared from crosslinked poly (N-isopropylacrylamide) (PNIPAAm). The swelling behavior of PNIPAAm is characterized by a low critical solution temperature (LCST). The LCST of a pure PNIPAAm is 32°C and the polymer chains collapse at a temperature higher than 32°C. This value can be varied by co-polymerization with a more hydrophobic or hydrophilic polymer, respectively [82–84], as shown in Fig. 2. In PNIPAAm gels modified via interpenetration with a second network [84–87] or by conjugation or grafting [88] the LCST remains almost constant. Hitotsu *et al.* [89] studied the LCST of crosslinked PNIPAAm and determined that it could be increased by mixing small amounts of ionic copolymers in the gels. Beltran *et al.* [90] also studied PNIPAAm gels containing ionic co-monomers and observed similar results.

Many theories have been proposed to explain the LCST behavior in PNIPAAm gels [91]. Hydrophobic interactions and hydrogen bonding are major contributors to the LCST behavior [92]. The hydrophobic interactions arise between non-polar molecules in water. Up to a certain temperature, the hydrophobic group of a polymer

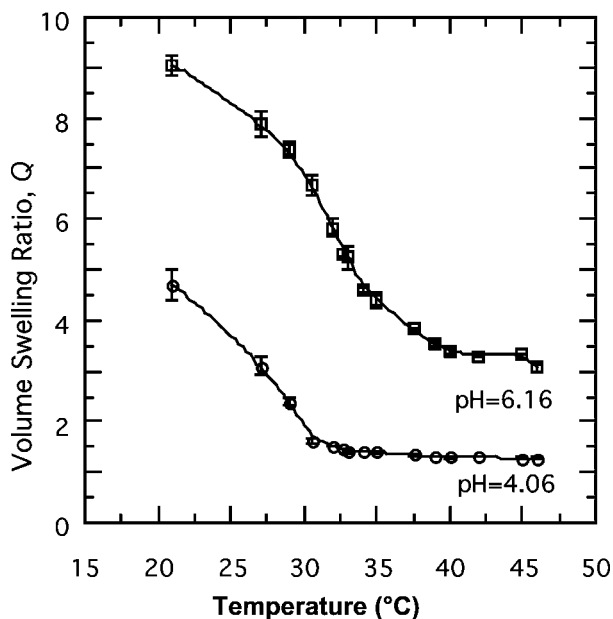


Figure 2. Equilibrium swelling behavior as a function of temperature at pH 4.06 (○) and pH 6.16 (□) for PMAA/PNIPAAm IPNs containing 70 mol% PNIPAAm.

chain is shielded by water molecules, which are arranged in a pattern to form a cage around the functional group. When the temperature is increased, this cage of immobile water molecules is partially lost and the protection of the hydrophobic groups becomes weakened. This may be the reason why hydrophobic interactions increase as the temperature is increased.

Hoffman [93] proposed the application of PNIPAAm and its copolymers for temperature-modulated drug release by gel ‘squeezing’ and surface regulation. In the bulk squeezing system, the drug that is distributed evenly inside the matrix is squeezed out of the system due to the deswelling of the hydrogel as a result of increasing the temperature of the environment above the volume phase transition temperature. In the surface regulation system, the swelling ratio of the skin layer is increased as the temperature of the system is lowered below the volume phase transition temperature and, hence, the drug molecules are able to diffuse through the skin layer. Clearly, these types of hydrogels exhibit all the desirable characteristics of coatings, layers or particles in formulations leading to multiple dosing devices.

Chen and Hoffman [94] prepared P(NIPAAm-g-AA) gels, which exhibited temperature- and pH-sensitive behavior. These gels could respond rapidly to both temperature and pH changes. The temperature- and pH-dependent swelling behavior was more pronounced in graft co-polymers than in random co-polymers containing similar amounts of pH- and temperature-sensitive components.

Okano and associates [95–102] developed comb-type graft hydrogels of PNIPAAm. The main chain of the crosslinked PNIPAAm contained low-molecular-

weight grafts of PNIPAAm. Under conditions of gel collapse (above the LCST), hydrophobic regions were developed in the pores of the gel resulting in a rapid collapse. These materials had the ability to collapse from a fully swollen conformation in less than 20 min, while comparable gels that did not contain graft chains required up to a month to fully collapse.

Inoue *et al.* [103] synthesized hydrogels grafted with oligomers with two different LCST. The oligomers of choice were carboxy-terminated oligo NIPAAm, oligo(*N*-vinylcaprolactam) (VCL) and random co-oligomers of NIPAAm and acrylamide. The resulting hydrogels showed two volume phase transition temperatures, corresponding to the LCSTs of the grafted side chains of oligo-NIPAAm and oligo-VCL. They explored the possibility of applying these hydrogels to release drugs as a function of temperature. Conjugates of PNIPAAm with various enzymes have also been reported [104, 105]. Ding *et al.* [104] synthesized conjugates of oligomer of PNIPAAm and trypsin. These conjugates were soluble in solution and could be catalyzed by enzymatic reactions. They could then be separated from the solution by thermal precipitation. The recovery of the conjugates by thermal precipitation was highly efficient (more than 95%), even after 14 cycles through the LCST. The enzyme conjugates were found to be more stable than the native trypsin in both solution and precipitated state.

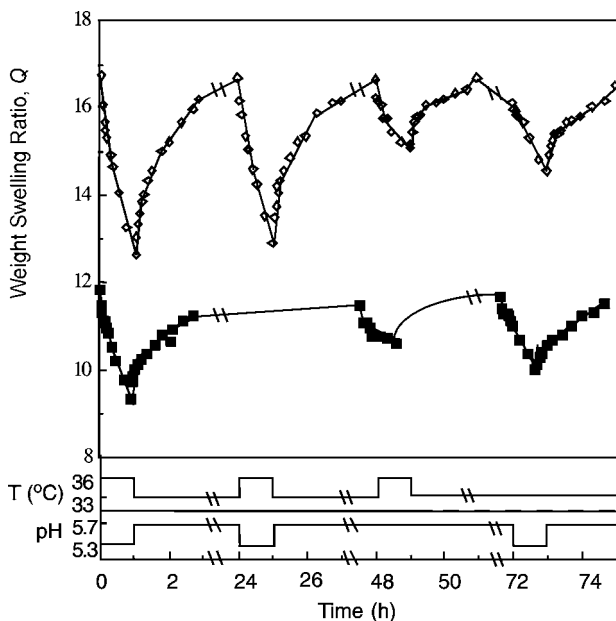


Figure 3. Weight swelling ratio of crosslinked P(NIPAAm-co-MAA) hydrogel samples containing 84 (■) and 88 (◇) mol% NIPAAm, placed successively in buffered solutions of pH 5.3 at 36°C and pH 5.7 at 33°C. Samples were equilibrated in their swollen state at pH 5.7 and 33°C, then placed for 1 h at pH 5.3 and 36°C, and allowed to return to their equilibrium swollen state at pH 5.7 and 33°C for several hours.

Serres *et al.* [106] and Vakkalanka *et al.* [79] developed hydrogels of PNIPAAm and PAA which were able to effectively release the protein drug, e.g. calcitonin, in response to temperature and pH changes. They prepared block copolymers of PNIPAAm and PMAA, which had the ability to respond to both temperature and pH (see Fig. 3). Using these materials, they were effectively able to modulate the release kinetics of streptokinase (see Fig. 4). Clearly, such systems would respond to minute changes of blood pH during initial clot formation triggering streptokinase release only when needed.

An efficient application of these ideas to the development of chronotherapeutic devices for GHRH release was discussed recently by us [107–109]. Micro- or nanoparticulate devices composed of hydrogels or containing hydrogel components and exhibiting thermosensitivity were incorporated in these devices. A key structural feature of these microcapsules was their composite coating system consisting of nanoparticles with crosslinked PNIPAAm shells dispersed in a thermo-insensitive ethylcellulose matrix. At low temperatures, permeability of the membranes was low because of absence of void formation by the swollen PNIPAAm shells in the microcapsule membrane, leading to low release rate of drugs. At high temperatures, the PNIPAAm shells could collapse. We have expanded on recent studies by Leobandung *et al.* [108, 109].

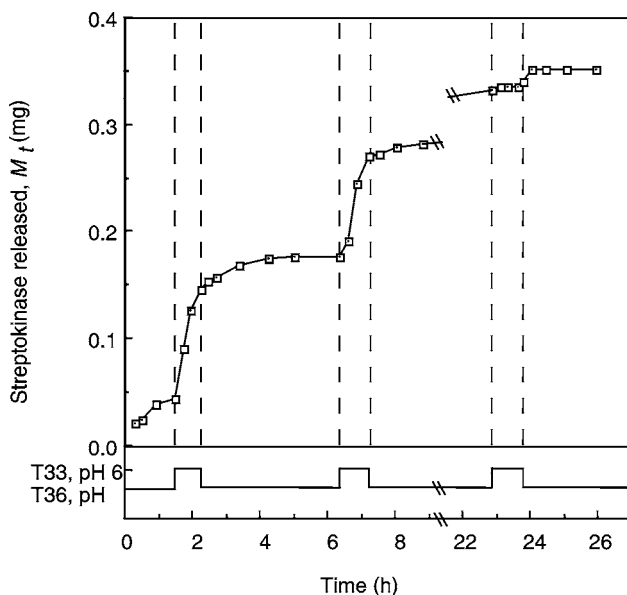


Figure 4. Streptokinase released in buffered solutions from P(NIPAAm-co-MAA) hydrogels containing 84 mol% NIPAAm upon change in temperature from 36 to 33°C and pH from 5.0 to 6.0 for 1 h and back to 36°C and pH 5 for a longer time interval.

Other stimuli-sensitive hydrogels

Stimuli other than pH and temperature can trigger drug release from a polymer carrier. Physical stimuli, such as light, magnetic field [110], electric current [111] and ultrasound [112], can be applied to the systems externally. Chemical stimuli [113], certain chemical substances and biological compounds [114] can be implemented. In some cases, their effective applications can be found in engineering rather than pharmaceutical fields. For example, carrier osmosis, dissolution or melting can be used for predetermined release of drugs.

Versatile stimuli-sensitive controlled release systems can be fabricated, provided that hydrogels are well designed to alter their configuration in response to these stimuli. Meanwhile, elucidation of the release mechanisms relies on more complicated mass transport phenomena over conventional diffusion-regulating systems is an important issue in the substantial application of stimuli-sensitive controlled release devices. In this context, Lavon and Kost [111] examined mass transport enhancement in non-erodible polymeric controlled release systems by ultrasound for a better understanding of the ultrasound-enhancing drug release phenomenon. Hsu and Block [112] studied electrokinetic phenomena in three types of neutral and anionic gels, such as agarose, agarose-carbomer 934P and agarose-xanthan gum, under an applied electric current for electrically-modulated drug delivery. As expected, the electrical current and the gel concentration could influence both syneresis and drug migration. These findings may give a useful insight in the application of hydrogels to transdermal delivery assisted by electroporation, iontophoresis or sonophoresis.

Analyte-sensitive hydrogels. Probably the most promising controlled release systems for chronobiological and chronotherapeutic drug delivery are those that can respond to increased concentration of specific biological compounds in the body. By processes belonging to the general category of molecularly recognitive networks such carriers can recognize undesirable compounds produced at certain intervals (e.g. glucose) and release associated therapeutic agents. For this purpose, several classes of hydrogels that respond to specific molecules (analyte-sensitive hydrogels) have been studied. For example, calcium-responsive bioerodible drug-delivery systems were devised by Goldbart and Kost [113]. These systems are composed of a starch-cellulose matrix containing α -amylase, whose activity is regulated by calcium. The principle relies on calcium responsiveness based on the mechanism that α -amylase in its non-active form is incorporated into a matrix (composed of starch and the matrix), thus responds to calcium which causes the non-active α -amylase to become active.

Fascinating stimuli-sensitive hydrogel-based drug-delivery systems were proposed by Miyata *et al.* [114, 115] who designed novel reversibly antigen-responsive hydrogels based on a unique idea. Hydrogels from acrylamide (AAm)-based semi-IPN consisting of antibody-grafted linear PAAm and crosslinked PAAm were grafted with the corresponding antigen. In the absence of free antigen, the hy-

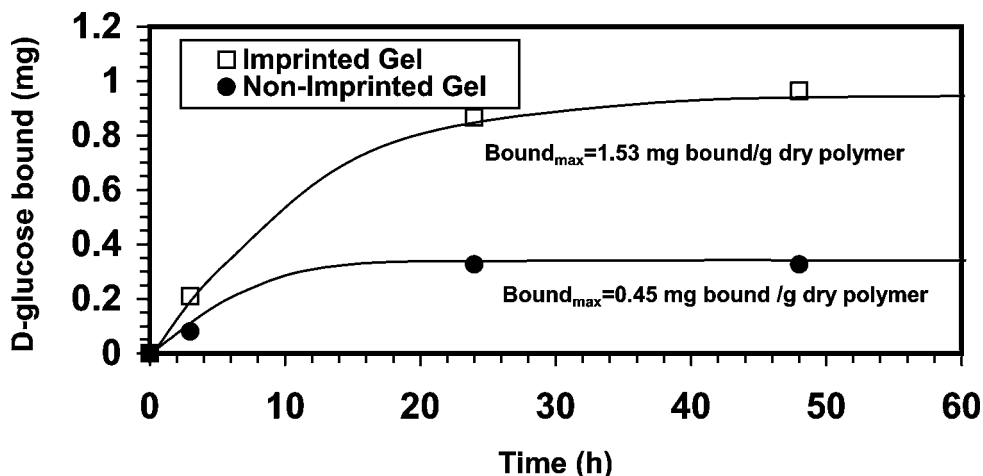


Figure 5. Kinetic D-glucose binding study in water. Acrylamide-PEG 200 DMA co-polymers of 67% crosslinking ratio prepared in DMSO ($T = 24^{\circ}\text{C}$).

drogel can shrink due to the intra-chain antigen–antibody binding in the polymer network, while in the presence of the free antigen it swells because of dissociation of the intra-chain binding by exchange of the grafted antigen for free antigen. This swelling/shrinking process was shown to be reversible. Due to this property, the hydrogel membrane allowed antigen-responsive change of hemoglobin permeation in response to stepwise changes in the antigen concentration.

A striking feature of certain stimuli-sensitive hydrogels is the specificity of molecular recognition. Using novel methods of biomimetic configurational imprinting, we have been able to prepare stimuli and analyte-sensitive hydrogels for molecular recognition [116]. Generally, co-polymer films of differing composition of template, crosslinker and functional monomer were synthesized in an appropriate type and amount of solvent via UV free-radical polymerization in a nitrogen atmosphere. Specific examples of D-glucose-imprinted polymers were shown although these techniques are applicable to other biologically significant molecules, in which hydrogen bonding, hydrophobic, or ionic contributions will direct recognition (see also Fig. 5).

We have been successful in synthesizing novel glucose-binding gels based on non-covalent interactions (hydrogen bonding, hydrophobic interactions) formed via CBIP techniques within polar media (protic solvent (water), water/ethanol and aprotic solvent (dimethylsulfoxide, DMSO)). Glucose has a molecular weight of 180 g/mol, and in solution glucose mutarotates between two solution conformations: alpha (25–30%) and beta (65–75%) position of the carbon 1 hydroxyl group at equilibrium.

Heteropolymer gels were prepared using: methacrylic acid (MAA)–ethylene glycol dimethacrylate (EGDMA) co-polymers prepared in ethanol/water; acrylamide (Aam)-poly(ethylene glycol) 200 dimethacrylate (PEG 200 DMA) co-

polymers prepared in water; Aam-vinylbenzene(VB)-EGDMA co-polymers prepared in ethanol/water; Aam-EGDMA co-polymers prepared in ethanol/water; Aam-PEG 200 DMA co-polymers prepared in DMSO; and acrylic acid (AA)-PEG 200 DMA co-polymers prepared in DMSO. Polymerizations occurred between glass microscope slides ($75 \times 50 \times 1$ mm, Fisher Scientific, Pittsburgh, PA, USA) using 0.5 mm and 0.035 mm Teflon[®] spacers in a nitrogen atmosphere at a UV intensity of 15.0 mW/cm^2 (UltraCure 100, EFOS, Ontario, Canada). The polymerization time was 15 min.

Polymers were either allowed to dry at ambient conditions and then were crushed and sieved (range from 300 to less than $150 \mu\text{m}$) or were cut into various diameter discs using a cork borer. If processed into discs, polymers were placed in deionized water for a period of 24 h, carefully separated from the slides, and then were cut into various diameter discs. Discs/particles were then placed in 50-ml conical tubes and placed on a rotating mixer and resuspended within multiple wash steps.

Incorporating higher-molecular-weight crosslinking monomers (longer chain length) without incorporating a correspondingly increased functional monomer chain length had a profound effect on the recognitive properties of the imprinted gels. Polymers consisting of increased dimethacrylate chain sizes (EGDMA, polyethylene glycol dimethacrylate (PEG 200 DMA, etc.) and increased functional monomer chain sizes were prepared with varying degrees of crosslinking content and analyzed regarding affinity and specificity.

Such systems have been used [58, 116] to release drugs or proteins after recognition of the undesirable biological and 'triggering' of a release mechanism for the drug. While still at their infancy, they have been shown to have great potential in revolutionizing drug treatment due to their versatility and mimicking of the natural response of our body.

CONCLUSIONS

In conclusion, stimuli-sensitive hydrogels are excellent candidates for chronotherapeutic applications because they can respond to external triggers. The purpose of this review was to show the importance of circadian rhythms and their impact on everyday life and medicine, with respect to clinical chronobiology and chronotherapeutics. Thirty or so medical conditions were identified that display predictable 24-h variation in pathophysiology, symptom intensity and mortal events. Overall, this suggests that delivering medication in synchrony with rhythms in pathophysiology or host tolerance or both can enhance treatment outcomes and compliance. The tools to design such formulations are based on judicious selection of hydrogels with appropriate response to surrounding fluids.

Acknowledgements

Results from our laboratories reported in this work (and especially the studies of Mark Byrne, Zachary Hilt and W. L.) have been supported by multiple grants from the National Institutes of Health and the National Science Foundation.

REFERENCES

1. S. W. Sanders, A. L. Bishop and J. G. Moore, *Chronobiol. Int.* **8**, 267 (1991).
2. B. Lemmer, *Pharm. Res.* **33**, 107 (1996).
3. R. J. Konopka and S. Benzer, *Proc. Natl. Acad. Sci. USA* **68**, 2112 (1971).
4. T. A. Bargiello, F. R. Jackson and M. W. Young, *Nature* **312**, 752 (1984).
5. M. P. Myers, K. Wager-Smith, C. S. Wesley, M. W. Young and A. Seghal, *Science* **270**, 805 (1995).
6. A. Seghal, A. Rothenfluh-Hilfiker, M. Hunter-Ensor, Y. Chen, M. P. Myers and M. W. Young, *Science* **270**, 808 (1995).
7. N. Gekakis, L. Saez, A. Delahaye-Brown, M. P. Myers, A. Seghal, M. W. Young and C. J. Weitz, *Science* **270**, 811 (1995).
8. J. P. Barnes, *Am. J. Med.* **79**, 5 (1985).
9. J. P. Barnes (Ed.), in: *Chronopharmacology—Cellular and Biochemical Interactions*, pp. 53–63. Marcel Dekker, New York, NY (1989).
10. M. H. Smolensky, in: *Chronopharmacology—Cellular and Biochemical Interactions*, J. P. Barnes (Ed.), pp. 65–113. Marcel Dekker, New York, NY (1989).
11. M. H. Smolensky, *Am. J. Hypertens.* **9**, 11 (1996).
12. M. W. Millar-Craig, C. N. Bishop and E. B. Raftery, *Lancet* **i**, 795 (1978).
13. G. A. Mansoor, E. J. McCabe and W. B. White, *J. Hypertens.* **12**, 703 (1994).
14. W. B. White, *Am. J. Hypertens.* **9**, 29S (1996).
15. K. Nakamura, J. Oita and T. Yamaguchi, *Stroke* **26**, 1373 (1995).
16. R. E. Burns, *Cancer Res.* **41**, 2795 (1981).
17. F. Lévi, M. Benavides, C. Chevelle, F. LeSaunier, F. Bailleul, J. L. Misset, C. Regensberg, J. M. Vannetzel, A. Reinberg and G. Mathé, *J. Clin. Oncol.* **8**, 705 (1990).
18. Z. Darzynkiewicz, B. Williamson, E. Carswell and L. J. Old, *Cancer Res.* **44**, 83 (1984).
19. N. A. Boughattas, F. Lévi, C. Fournier, G. Lemaigre, A. Roulon, B. Hecquet, G. Mathé and A. Reinberg, *Cancer Res.* **49**, 3362 (1989).
20. N. A. Boughattas, F. Lévi, B. Hecquet, G. Lemaigre, A. Roulon, C. Fournier and A. Reinberg, *Toxicol. Appl. Pharmacol.* **96**, 233 (1988).
21. R. E. Burns, *Cancer Res.* **41**, 2915 (1981).
22. W. J. M. Hrushesky, R. Borch and F. Lévi, *Clin. Pharmacol. Ther.* **32**, 330 (1982).
23. J. A. Sinkule, K. Y. Choi, R. Von Roemeling and L. Langevin, *Annu. Rev. Chronopharmacol.* **3**, 215 (1986).
24. M. L. Hartman, A. Iranmanesh and M. O. Thorner, *Am. J. Hum. Biol.* **5**, 603 (1993).
25. M. T. Bluet-Pajot, J. Epelbaum, D. Gourdjji, C. Hammond and C. Kordon, *Cell. Mol. Neurobiol.* **18**, 101 (1998).
26. K. A. Lacey and J. M. Parkin, *Lancet* **i**, 42 (1974).
27. G. V. Vimpani, A. F. Vimpani, G. P. Lidgard, E. H. D. Cameron and J. W. Farquhar, *Br. Med. J.* **2**, 427 (1977).
28. R. J. Rona and J. M. Tanner, *Arch. Dis. Childhood* **52**, 197 (1977).
29. E. Corpas, S. M. Harman and M. R. Blackman, *Endocr. Rev.* **14**, 20 (1993).
30. E. Ghigo, E. Arvat, G. Aimaretti, F. Broglio, R. Giordano and F. Camanni, *Clin. Endocrinol. Metab.* **12**, 341 (1998).

31. M. O. Thorner, I. M. Chapman, B. G. Gaylinn, S. S. Pezzoli and M. L. Hartman, *Rec. Progr. Horm. Res.* **52**, 215 (1997).
32. D. C. Hindmarsh, D. R. Matthews, I. Stratton, P. J. Pringle and C. G. Brook, *Clin. Endocrinol.* **36**, 165 (1992).
33. J. J. Chipman, A. F. Attanasio, M. A. Birkett, P. C. Bates, S. Webb and S. W. Lamberts, *Clin. Endocrinol.* **46**, 473 (1997).
34. J. M. Chan, M. J. Stampfer, E. Giovannucci, P. H. Gann, J. Ma, P. Wilkinson, C. H. Hennekens and M. Pollak, *Science* **279**, 563 (1998).
35. R. C. Cuneo, S. Judd, J. D. Wallace, D. Perry-Keene, H. Burger, S. Lim-Tio, B. Strauss, J. Stockigt, D. Topliss, F. Alford, L. Hew, H. Bode, A. Conway, D. Handelsman, S. Dunn, S. Boyages, N. W. Cheung and D. Hurley, *J. Clin. Endocrinol. Metab.* **83**, 107 (1998).
36. M. L. Vance, D. L. Kaiser, W. S. Evans, M. O. Thorner, R. Furanetto, J. Rivier, W. Vale, G. Perisutti and L. A. Frohman, *J. Clin. Endocrinol. Metab.* **60**, 370 (1985).
37. K. Y. Ho and D. M. Hoffman, *Horm. Res.* **40**, 80 (1993).
38. S. Reichlin, *Endocrinology* **67**, 760 (1960).
39. S. Reichlin, *Endocrinology* **69**, 225 (1961).
40. R. R. Deuben and J. Meites, *Endocrinology* **74**, 408 (1964).
41. J. Rivier, J. Spiess, M. Thorner and W. Vale, *Nature* **200**, 276 (1982).
42. R. Guillemain, P. Brazeau, P. Bohlen, F. Esch, N. Ling and W. B. Wehrenberg, *Science* **218**, 585 (1982).
43. L. A. Frohman and J. Jansson, *Endocr. Rev.* **7**, 223 (1986).
44. B. D. Gaylinn, J. K. Harrison, J. R. Zysk, C. E. Lyons, K. R. Lynch and M. O. Thorner, *Mol. Endocrinol.* **7**, 77 (1993).
45. M. Barinaga, G. Yamamoto, C. Rivier, W. Vale, R. Evans and M. G. Rosenfeld, *Nature* **306**, 84 (1983).
46. M. Kato, J. Hoyland, S. K. Sikdar and W. T. Mason, *J. Physiol.* **447**, 171 (1992).
47. M. Kato and Y. Sakuma, *Endocrinology* **138**, 5096 (1997).
48. N. C. Guarineau, X. Bonnefont, L. Stoeckel and P. Mollard, *J. Biol. Chem.* **273**, 10389 (1998).
49. E. Kochman and K. Kwarecki, *Acta Physiol. Polon.* **35**, 265 (1984).
50. A. Lesault, B. Elchinger and B. Desbals, *Horm. Metab. Res.* **23**, 515 (1991).
51. R. J. Feuers, J. D. Hunters, T. H. Tsai, S. S. Cardoso and L. E. Scheving, *Prog. Clin. Biol. Res.* **341A**, 529 (1990).
52. C. Simon, *Horm. Res.* **49**, 185 (1998).
53. A. Kalsbeek and J. H. Strubbe, *Physiol. Behav.* **63**, 553 (1998).
54. A. J. Scheen and E. Van Cauter, *Horm. Res.* **49**, 191 (1998).
55. C. Schöneich, M. J. Hageman and R. T. Borchardt (Eds), in: *Controlled Drug Delivery Challenges and Strategies*, pp. 205–228. American Chemical Society, Washington, DC (1997).
56. H. Thurow and K. Geisen, *Diabetologia* **27**, 212 (1984).
57. M. Hagelocher and R. Pearlman, in: *4th Annual Meeting of the AAPS*, Atlanta, GA, Abstract BT219 (1989).
58. N. A. Peppas and M. E. Byrne, *Bull. Gattefossé* **96**, 23 (2003).
59. N. A. Peppas and P. Colombo, *J. Control. Rel.* **45**, 35 (1997).
60. N. A. Peppas, *J. Bioact. Compat. Polym.* **6**, 241 (1991).
61. A. Katchalsky and I. Michaeli, *J. Polym. Sci.* **15**, 69 (1955).
62. L. Brannon-Peppas and N. A. Peppas, *Chem. Eng. Sci.* **46**, 715 (1991).
63. A. Katchalsky, *Experimentia* **5**, 319 (1949).
64. A. Katchalsky, S. Lifson and H. Eisenberg, *J. Polym. Sci.* **7**, 571 (1951).
65. T. Tanaka, *Polymer* **20**, 1404 (1979).
66. L. Brannon-Peppas and N. A. Peppas, in: *Absorbent Polymer Technology*, L. Brannon-Peppas and R. Harland (Eds), pp. 67–75. Elsevier, Amsterdam (1990).

67. W. Oppermann, in: *Polyelectrolyte Gels: Properties, Preparation, and Applications*, ACS Symposium Series No. 480, pp. 159–170. American Chemical Society, Washington, DC (1992).
68. A. B. Scranton, B. Rangarajan and J. Klier, *Adv. Polym. Sci.* **120**, 1 (1995).
69. R. Skouri, F. Schoessler, J. P. Munch and S. J. Candau, *Macromolecules* **28**, 197 (1995).
70. M. Rubinstein, R. H. Colby, A. V. Dobrynin and J. F. Joanny, *Macromolecules* **29**, 398 (1996).
71. U. P. Schroder and W. Opperman (Eds), in: *The Physical Properties of Polymeric Gels*, pp. 19–38. Wiley, New York, NY (1996).
72. E. Kramarenko and A. R. Khoklov, *Macromolecules* **30**, 3383 (1997).
73. J. Ricka and T. Tanaka, *Macromolecules* **17**, 2916 (1984).
74. I. Ohmine and T. Tanaka, *J. Chem. Phys.* **77**, 5725 (1992).
75. A. R. Khare and N. A. Peppas, *Biomaterials* **16**, 559 (1995).
76. B. A. Firestone and R. A. Siegel, *Polym. Commun.* **29**, 204 (1988).
77. R. A. Siegel and B. A. Firestone, *Macromolecules* **21**, 3254 (1988).
78. J. M. Cornejo-Bravo and R. A. Siegel, *Biomaterials* **17**, 1187 (1996).
79. S. K. Vakkalanka, C. S. Brazel and N. A. Peppas, *J. Biomater. Sci. Polymer. Edn* **8**, 119 (1996).
80. S. Saito, M. Konno and H. Inomata, *Adv. Polym. Sci.* **109**, 201 (1993).
81. T. Tanaka, *Phys. Rev. Lett.* **40**, 820 (1978).
82. Y. H. Bae, T. Okano and S. W. Kim, *Pharm. Res.* **8**, 531 (1991).
83. A. Gutowska, Y. H. Bae and S. W. Kim, *J. Control. Rel.* **22**, 95 (1992).
84. A. S. Hoffman, A. Afrassiabi and L. C. Dong, *J. Control. Rel.* **4**, 213 (1986).
85. K. Mukae, Y. H. Bae, T. Okano and S. W. Kim, *Polym. J.* **22**, 206 (1990).
86. A. Gutowska, Y. H. Bae, H. Jacobs, J. Feijen and S. W. Kim, *Macromolecules* **27**, 4167 (1994).
87. J. Zhang and N. A. Peppas, *Macromolecules* **33**, 102 (2000).
88. J. P. Chen, H. J. Yang and A. S. Hoffman, *Biomaterials* **11**, 625 (1990).
89. S. Hirotsu, Y. Hirokawa and T. Tanaka, *J. Chem. Phys.* **87**, 1392 (1987).
90. S. Beltran, J. P. Baker, H. H. Hooper, H. W. Blanch and J. M. Prausnitz, *Macromolecules* **24**, 549 (1991).
91. H. G. Schild, *Progr. Polym. Sci.* **17**, 163 (1992).
92. Y. Li and T. Tanaka, *Annu. Rev. Mater. Sci.* **22**, 243 (1992).
93. A. S. Hoffman, *J. Control. Rel.* **6**, 297 (1987).
94. G. H. Chen and A. S. Hoffman, *Nature* **373**, 49 (1995).
95. Y. Kaneko, S. Nakamura, K. Sakai, A. Kikuchi, T. Aoyagi, Y. Sakurai and T. Okano, *Polym. Gels Network* **6**, 333 (1998).
96. R. Yoshida, K. Uchida, Y. Kaneko, K. Sakai, A. Kikuchi, Y. Sakurai and T. Okano, *Nature* **374**, 240 (1995).
97. Y. Kaneko, K. Saki, A. Kikuchi, Y. Sakurai and T. Okano, *Macromol. Symp.* **109**, 41 (1996).
98. H. A. von Recum, S. W. Kim, A. Kikuchi, M. Okuhara, Y. Sakurai and T. Okano, *J. Biomed. Mater. Res.* **40**, 631 (1998).
99. H. Sakai, Y. Doi, T. Okano, N. Yamada and Y. Sakurai (Eds), in: *Advanced Biomaterials in Biomedical Engineering and Drug Delivery Systems*, pp. 229–230. Springer, Tokyo (1996).
100. T. Okano, N. Yamada, H. Sakai and Y. Sakurai, *J. Biomed. Mater. Res.* **27**, 1243 (1993).
101. T. Okano, N. Yamada, M. Okuhara, H. Sakai and Y. Sakurai, *Biomaterials* **16**, 297 (1995).
102. A. Kikuchi, M. Okuhara, F. Karikusa, H. Sakai, Y. Sakurai and T. Okano, in: *Transactions of 5th World Biomaterials Congress Vol. I*, p. 907. Transactions, Toronto (1996).
103. T. Inoue, G. Chen, K. Nakamae and A. S. Hoffman, *Polym. Gels Networks* **5**, 561 (1997).
104. Z. Ding, G. Chen and A. S. Hoffman, *J. Biomed. Mater. Res.* **39**, 498 (1998).
105. M. Matsukata, T. Aoki, K. Sanui, N. Ogata, A. Kikuchi, Y. Sakurai and T. Okano, *Bioconj. Chem.* **7**, 96 (1996).
106. A. Serres, M. Baudys and S. W. Kim, *Pharm. Res.* **13**, 196 (1996).
107. H. Ichikawa and Y. Fukumori, *J. Control. Rel.* **63**, 107 (2000).

108. W. Leobandung, H. Ichikawa, Y. Fukumori and N. A. Peppas, *J. Control. Rel.* **80**, 357 (2002).
109. W. Leobandung, H. Ichikawa, Y. Fukumori and N. A. Peppas, *J. Appl. Polym. Sci.* **87**, 1678 (2003).
110. M. Zrínyi, D. Szabó and H.-G. Kilian, *Polym. Gels Networks* **6**, 441 (1998).
111. I. Lavon and J. Kost, *J. Control. Rel.* **54**, 1 (1998).
112. C.-S. Hsu and L. H. Block, *Pharm. Res.* **13**, 1865 (1996).
113. R. Goldbart and J. Kost, *Pharm. Res.* **16**, 1483 (1999).
114. T. Miyata, N. Asami and T. Uragami, *Nature* **399**, 765 (1999).
115. T. Miyata, N. Asami and T. Uragami, *Macromolecules* **32**, 2082 (1999).
116. M. E. Byrne, E. Oral, J. Z. Hilt and N. A. Peppas, *Polym. Adv. Technol.* **13**, 798 (2002).