ELSEVIER

Advanced Drug Delivery Reviews 31 (1998) 197–221

advanced drug delivery reviews

# Temperature-responsive gels and thermogelling polymer matrices for protein and peptide delivery

Lev E. Bromberg[1,*], Eyal S. Ron

*Sensei Pharmaceutical, 7 Coach Road, Lexington, MA 02173, USA*

**Abstract**

The explosive development of protein and peptide drugs has ushered in a great need for effective delivery systems for such drugs. Two decades of studies of protein delivery from polymeric systems has revealed the great potential of gels that respond to environmental stimuli, such as temperature. Protein and peptide release can be engineered to occur in a pulsatile mode. The mechanism of this release is greatly affected by polymer design. 'Intelligent' amphiphilic copolymers emerge as a novel trend in the application of thermodynamically stable self-assembling lyophilic colloids to protein and peptide delivery.


*Keywords:* Pluronic-*g*-poly(acrylic acid) conjugates; Pluronic surfactants; Poly(ethylene oxide) and poly(propylene oxide) triblock copolymers; Protein and peptide delivery; Temperature-sensitive polymers

**Contents**



## 1. Introduction

Rapid progress in recombinant DNA technology has resulted in the ready availability of a wide

*Corresponding author.

[1]Current address: MediSense, Inc., An Abbott Laboratories Company, 4A Crosby Drive, Bedford, MA 01730, USA.

0169-409X/98/$19.00 
*PII* S0169-409X(97)00121-X

variety of protein- and peptide-based drugs targeting poorly controlled diseases [1,2]. Several hundred protein drugs are currently undergoing clinical trials [2,3]. It has been understood for more than 20 years [4] that large proteins could be delivered slowly and continuously from polymeric systems, and since that time an explosive growth in the number of publications in the field of controlled delivery of proteins and peptides has occurred. Parenteral and, to a certain extent, oral routes of administration of proteins and peptides were dominated by micro- and nano-particulate systems where the drug is encapsulated in a solid polymer [5–8]. The first Food and Drug Administration (FDA) approved system was an analog of lutenizing hormone-releasing hormone (LHRH), leuprolide acetate, encapsulated into microspheres composed of lactic acid–glycolic acid copolymer (Lupron Depot) [9]. It is quite significant that this progress has not been matched by comparable advances in development and formulation of non-parenteral peptide and protein drug delivery systems. It appears that the bioavailability of peptides and proteins from non-oral mucosal routes is in general poor when compared with the parenteral route. This is due to several basic barriers, including highly selective epithelia that exclude macromolecules, the presence of proteolytically destructive multistage enzyme systems, as well as non-enzymatic clearance mechanisms [10]. On the other hand, unlike other drug formulations, protein formulations are susceptible to loss of both native structure (through cleavage of peptide bonds and destruction of amino acid residues, e.g. proteolysis, oxidation, deamidation, and β-elimination) and conformation (from the disruption of noncovalent interactions, e.g. aggregation, precipitation, and adsorption) [11–13]. Mechanisms leading to protein destabilization in drug delivery formulations have been recently reviewed [14]. Among other strategies used to circumvent the poor bioavailability of proteins and peptides, numerous penetration enhancers were studied as a means to affect and, to a certain extent, alter the mechanism with which proteins cross mucosal membranes [15,16]. Depending on the type of epithelia that a drug should ultimately cross in order to reach its intended site of action, penetration enhancers may target transcellular or paracellular routes [17]. In some cases, like in the case of poly(ethylene oxide)-modified proteins [18], not only is penetration enhanced, but also the stability of the protein formulations [19]. Optimization of the delivery system with respect to retention at the absorption site appears to be an alternative to traditional penetration enhancers [10]. Various dosage forms have been studied in this regard, including aqueous solutions [20], powders [21], microspheres [14,22], and gels [23–28]. In the present review, the authors focus on the gel systems only, as they can be designed to combine several potential ways to improve protein and peptide availability: (i) protect the drug from the hostile environment, including proteolytic enzymes and low pH (as in the stomach); (ii) control reaction of the body to the drug formulation; and, most importantly, (iii) control the release of the drug on demand by triggering changes in the gel structure by environmental stimuli. The latter possibility can be realized if a gel constituent is capable of responding to a stimulus (i.e. change in temperature, pH, solvent composition, etc.). In general, a temperature or other stimulus can be found that triggers volume phase transition in any gel [29,30]. However, the main subject of our study was safe ‘intelligent’ gel systems that can be safely applied in the human body. Herein, we concentrate on protein and peptide release using temperature-sensitive permanently crosslinked gels as well as polymer solutions that gel at body temperatures. The definition of the term ‘gel’ thus far has some ambiguity [31]. We will define a gel according to its phenomenological characteristics as a soft, solid-like material that consists of several components, one of which is a liquid, present in substantial quantity. In drug delivery, the term ‘hydrogel’ is typically reserved for polymeric materials that can absorb a substantial amount of water while maintaining a distinct three-dimensional structure [32].

## 2. Temperature-responsive permanently crosslinked gels

### *2.1. Hydrophobic effect and critical solution temperatures*

Covalently crosslinked temperature-sensitive gels are perhaps the most extensively studied class of

environmentally-sensitive polymer systems in drug delivery [32–45]. At least one component of the polymer system should possess temperature-dependent solubility in a solvent (i.e. water, with a few exceptions [46,47]). In order to obtain a hydrogel that dramatically changes its swelling degree in water, the gel constituents must be insoluble above or below a certain temperature, called the lower or upper critical solution temperature (LCST or UCST, respectively). For drug release applications, mainly LCST systems are relevant [34]. The phenomenon of polymer aggregation at LCST is thermodynamically similar to that causing temperature-induced protein folding [48–50]. Namely, the driving force for the aggregation is the entropy ($S$) of the two-phase polymer and water system, which is greater than in polymer solution [51]. Positive $\Delta S$ renders the temperature increase to contribute to the trend of the system to aggregate, as the positive enthalpy term $\Delta H$ is smaller than the entropy term and does not influence the spontaneous association to a great extent [52,53]. Under these circumstances the free energy of association ($\Delta G = \Delta H - T\Delta S$) is negative, and thus association is favorable. A schematic diagram of the hydrophobic association is given in Fig. 1.

It is interesting to note that although the requirement of a negative excess entropy of mixing for an LCST has been theoretically proven [54], the nature of the polymer–water interactions that make the water adjacent to the polymer more ordered than in the bulk, remains debatable. Whether it is purely a 'hydrophobic effect' [55–58], or hydrogen bonding [59,60], or both [61] that explain the LCST phenomenon, should be further clarified.

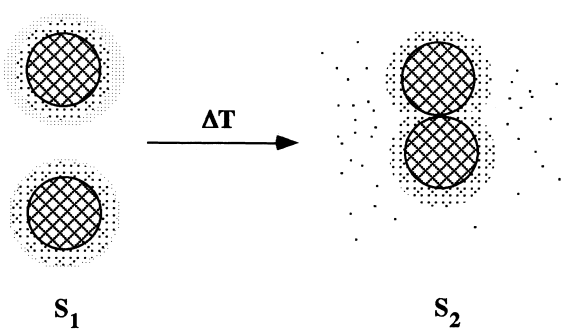



Fig. 1. Schematic representation of the hydrophobic interaction. State 1 corresponds to two non-associated molecules with the layers of structured water; state 2 represents aggregation when some water molecules leave structured layers for less structured bulk. $S_2 > S_1$. Adopted, with changes, from Ref. [53].

Once LCST is reached, individual polymer chains collapse prior to aggregation, increasing scattering of light in the solution, causing cloudiness [54]. The cloud point is followed by the appearance of two phases; one is composed of collapsed gel that has expelled most of its associated water, and the other is the water itself. This process is of paramount importance for the application of thermoresponsive gels in drug delivery.

### 2.2. 'On–off ' and pulsatile delivery concepts

The abrupt shrinking of temperature-responsive gels above the LCST resulting in such a drastic change in their swelling degree has called forth a rather extensive research effort directed at the application of gels to the controlled release of drugs, and of proteins in particular [33–45,62–66]. Several concepts of drug delivery via temperature-sensitive hydrogels have been proposed (Fig. 2).

Some of them originate from the work on revers-

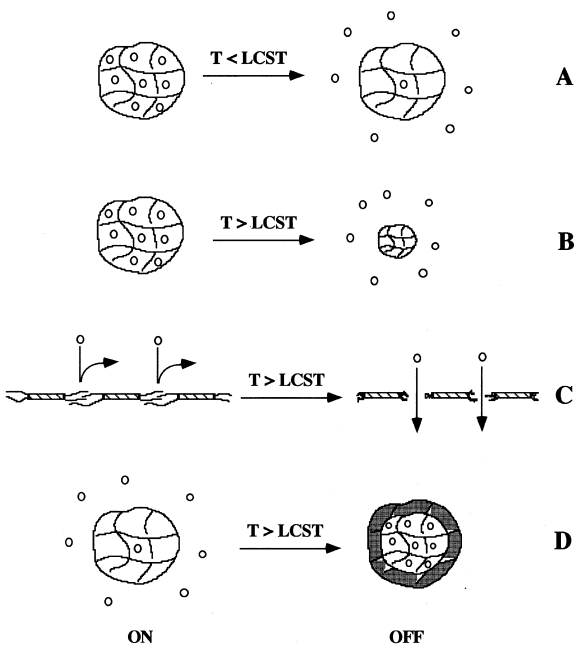



Fig. 2. Modes of drug delivery from temperature-sensitive hydrogels.

ible protein extraction by Cussler et al. [67,68]. When a hydrophilic drug is incorporated into a swollen gel, it can show a Fickian release below the LCST, the details of which depend on the swelling degree of the gel and the tortuosity of the pathway the drug must take (Fig. 2A) [33,54]. Conversely, a more hydrophobic drug can show Fickian diffusion from the collapsed gel above. Hoffman et al. [35,36] demonstrated that the rate of delivery of myoglobin and low-molecular-weight solutes can be altered when the temperature-triggered collapse of the gel occurs (Fig. 2B). If a drug is loaded below the LCST, it can be squeezed out above the LCST due to the pressure generated during gel collapse. A similar idea was realized with gels immobilized within porous membranes [69–71]. There, swollen gel blocks both diffusion and convection flow through the pores, and allows permeation when collapsed (Fig. 2C). Alternatively, if a gel is essentially heterogeneous, it may form a dense 'skin' layer of the collapsed component while the core remains swollen (Fig. 2D). This kind of gel structure allowed Okano et al. [64] to model an auto-feedback glucose–insulin system using pulsatile drug release. Finally, thermoresponsive gels allow for the control of enzymatic activity. Hoffman [62] presented a concept of immobilization of either the enzyme or substrate in the swollen gel resulting in turning the enzymatic reaction on and off below and above LCST due to diffusion limitations in the collapsed, dense polymeric phase.

### *2.3. Protein loading and release: Concepts and their limitations*

It is widely speculated that beyond the variety of the rate-controlling release mechanisms discussed above, the temperature-sensitive hydrogels can provide some versatility of formulation, a non-denaturing environment, and adjustable permeability for bioactive proteins [11,62–64,72]. Despite the variety of available methods of protein immobilization in gels [73], there are relatively few techniques of gel loading acceptable for drug delivery (Fig. 3) [74].

The first method involves mixing the drug with the appropriate monomer, crosslinker and initiator solution which then is allowed to polymerize, entrapping the drug within the formed matrix [74,75]. A modification of this method is to mix a drug with an already formed polymer and then crosslink it. Both techniques suffer from the possibility of side reactions that can denature the protein, as well as the inability to extract the sol fraction without also removing the protein [76]. Another, most widely used, approach is to allow a preformed purified gel to equilibrium swell in a drug-containing solution. The exclusion of large molecules, like proteins, from the hydrogel networks is a serious limitation of this approach, since loading levels achieved by solution sorption are often less than 0.1 wt% [72]. This limitation, which stems from steric repulsion forces between hydrated polymer coils and proteins, may be overcome if a dry gel network is allowed to swell in a non-aqueous protein solution, then dried and reswollen in water [77]. Application of non-aqueous solvents, which allows dramatically enhanced protein loading, is limited to a relatively few protein-dissolving organic solvents [78], unless protein solubility is enhanced by complexation of proteins with surfactants [79–81]. Yet another strategy was proposed by

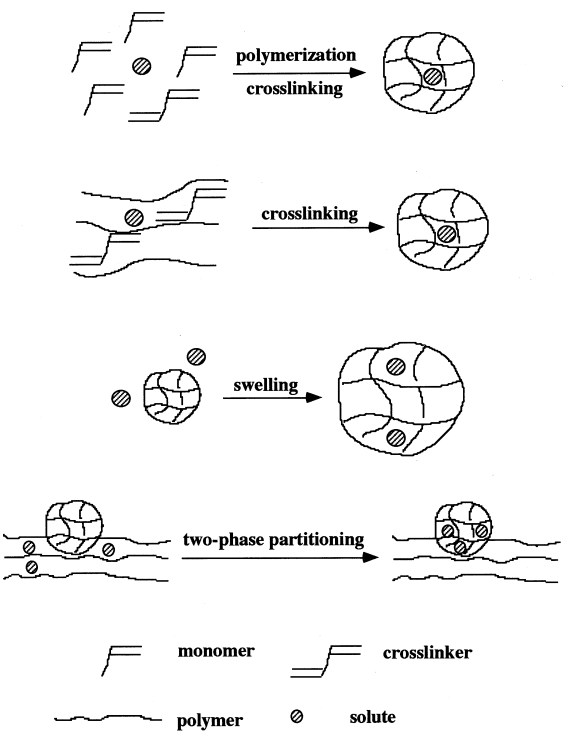



Fig. 3. Modes of gel loading with a protein.

Gehrke et al. [76,82] who achieved very high loading of proteins in gels by partitioning of proteins from polymer solutions of low affinity (i.e. poly(ethylene oxide) solutions) into the gel phase of higher affinity (such as dextran). The additional advantage of the latter technique is that the hydrogel plus any solutes loaded from low affinity solution enhance the bioactivity of the loaded proteins [11,82].

Typically, the loading of hydrogels with hydrophilic solutes, as well as their release, is considered to be a function of the gel swelling, absent significant polymer–solute interactions [74,83]. The swelling of gels that undergo volume phase transitions has been the subject of a large amount of work, both theoretical and experimental. Based on the Flory–Huggins mean-field approach [84,85], Tanaka and co-workers developed a mean-field theory for gels [86–89]. This theory regards swelling as a function of osmotic pressure in the gel which at equilibrium is equal to the osmotic pressure in the surrounding liquid. The total osmotic pressure ($\Pi$) is the sum of contributions of the mixing free energy ($\Pi_m$) and elastic free energy ($\Pi_{el}$), as well as the Donnan potential ($\Pi_{ion}$). Terms $\Pi_m$ and $\Pi_{ion}$ which reflect the tendency of the gel to swell, are balanced with the configurational (entropic) term $\Pi_{el}$ which reflects the elasticity of the network preventing the gel from dissolving. Some oversimplifications made in the classic theory (such as, for instance, Gaussian statistics of the polymer chains, which does not hold even for non-responsive gels [90]) were later corrected, which resulted in an improved equation of state [30]. The latter theory adequately explains swelling and volume phase transition in thermosensitive gels [91]. Other models that account for the features neglected in Flory–Huggins theory are the compressible lattice theory of Sanchez and Lacombe [92–94] applied to crosslinked gels by Cussler et al [95–97], as well as hydrogen bond 'interaction energies' by Prausnitz and co-workers [98–100].

Overall gel swelling represents the total volume of water absorbed per volume of dry polymer and is a measure of the 'free volume' available for diffusion of hydrophilic solutes, as reported by Yasuda et al. [101]. However, not only the overall volume fraction of water, but also its energetic state can influence the solute diffusion in gels [102,103]. Water in polymers can be either energetically identical to the bulk water ('free') or associated with the polymer chains ('bound'). The transport of hydrophilic solutes does not occur through the gels containing only bound water [104]. While the free volume theory can successfully predict transport of small hydrophilic solutes within gels which do not drastically change their structure upon a change in temperature [102,103], it overestimates by more than 30-fold the effective diffusion coefficient in the collapsed state of temperature-responsive gel [105]. The failure of the free volume theory to explain changes in the diffusion pattern upon volume transition in responsive gels is a very serious limitation, which is a result of the assumption that specific interactions between solute and polymer are absent [105].

It is clear that when the concepts of protein and peptide loading into and release from temperature-sensitive gels are discussed, interactions between proteins and polymers are to be considered. The nature and extent of these interactions control not only mechanisms of protein partitioning and diffusion, but also the ability of hydrogels to provide a safe environment for the proteins [106,107]. Many insights into protein–gel interactions come from the field of protein chromatography [53,108]. Although coulombic forces are mainly responsible for the binding between the gels and proteins (five out of the 20 amino acid side chains of proteins contain acidic or basic groups), in the case of uncharged thermoresponsive gels it is important to examine the hydrophobic properties of the proteins as well as hydrogen bonding. The hydrophobicity of protein surfaces is a function of the hydrophobicities of the exposed amino acids and backbone segments. Several approaches for the characterization of the protein hydrophobicity have been developed [109,110]. Importantly, temperature may change the structure of the protein and thus its hydrophobicity, since some hydrophobic groups may become exposed or buried within the protein globule [111].

Substantial affinity of the drug for the gel constituents due to the above interactions can potentially change the mechanism of transport through the gel, from diffusion in the volume of pores to transport along the polymer surface [112–117]. The latter depends on the effective partition coefficient between the volume phase and the polymer surface [116] and has been considered in detail [117]. The practical

implication of the change of the mechanism of diffusion might be that the increasing effective polymer concentration upon gel collapse increases the gel permeability toward the high affinity drug [105].

### *2.4. Gel materials and specific applications*

Chemically, the area of temperature-responsive gels has been dominated by *N*-alkylacrylamides with *N*-isopropylacrylamide (NIPA) being the most prominent example. Reviews of NIPA and its gel applications can be found in Refs. [54,118,119]. NIPA monomer and consequently polyNIPA show a very well defined LCST at about 32°C which can be shifted to body temperatures by the presence of ionic comonomers, as well as by formulating polyNIPA-based gels with salts, surfactants, etc. [54,120,121]. Although numerous poly(*N*-alkylacrylamides) and other polymers possess LCST [51,122], polyNIPA is quite unique with respect to the sharpness of its almost discontinuous transition, which is usually observed only with ionizable polymers [123]. *N*,*N*-Diethylacrylamide (DEAAm), with its LCST in the range 25–32°C, perhaps follows NIPA on a list of widely studied temperature-sensitive *N*-alkylacrylamide gel constituents [124–126]. Copolymers of NIPA or DEAAm and a variety of other *N*-alkylacrylamides with cross-linkers ranging from *N*,*N′*-methylenebis(acrylamide) and ethylene glycol dimethacrylate to *N*-methylolacrylamide have been studied [54]. The rule of thumb is that [54] the introduction of a more or less hydrophobic constituent would either lower or increase, respectively, the LCST of the resulting copolymer. Functionalization of polyNIPA-based gels with ionic groups, labels, hydrophobes, etc. was achieved by copolymerization of NIPA with other monomers [54,127–129], resulting in either macroscopic gels with desired shapes, such as beads, cylinders, sheets and fibers, or 'microgels' of sub-millimeter size [130,131]. Although studies of polyNIPA-based gels have not yielded the commercial introduction of a drug delivery device because of the reasons discussed below, they resulted in useful accumulation of knowledge, notably in the area of pulsatile protein release [132–135]. Peppas and co-workers [134,135] applied poly(*N*-isopropylacrylamide-co-methacrylic acid) gels crosslinked by ethylene glycol dimethacrylate to the delivery of antithrombotic agents, such as heparin, at the site of a blood clot, utilizing biological conditions to trigger drug release. Pulsatile delivery of streptokinase, a fibrinolytic enzyme used in the treatment of coronary thrombosis, was achieved [43,133,134]. Kim and co-workers [132,133] recently reported on the application of linear poly(*N*-isopropylacrylamide-*co*-butylmethacrylate-*co*-acrylic acid) terpolymers in the oral delivery of human calcitonin for potential treatment of osteoporosis and hypercalcemias. The polypeptide was solubilized in cold polymer solution below its LCST. Following addition of the aqueous polymer–polypeptide mixture into an oil bath kept above the LCST, the polymer precipitated in the form of beads, entrapping the polypeptide. The same technique was applied for loading insulin [133]. Although copolymers with somewhat higher acrylic acid content were much more efficient in terms of loading efficiency and in vivo stability of calcitonin than more hydrophobic copolymers, they were less efficient in in vitro sustained release due to fast swelling. Since this concept involves control of the polypeptide release by the rate of degradation of the beads, a very fine 'tuning' of the degradation in the desired part of the gastrointestinal tract is required.

Beyond 'traditional' copolymeric gel systems, temperature-responsive gels can be designed from an alloy composed of two independent interpenetrating polymer networks (IPNs), where one crosslinked network is intertwined with another [136,137]. Okano and co-workers [42,136] designed IPNs of independently crosslinked poly(acrylic acid) and poly(*N*,*N*-dimethylacrylamide), poly(ethylene oxide) and poly(*N*-acryloylpyrrolidine), poly(acrylamide-*co*-butyl methacrylate) and poly(acrylic acid), etc. that show temperature-dependent solubility changes due to the formation and dissociation of interpolymer hydrogen bonds. Tanaka et al. [138] pointed out that the cooperativity of breaking of hydrogen bonds is essential for the volume transition in IPNs as random and independent hydrogen bonds would not provide a sufficient driving force for the transition. Since hydrogen bonding is probably the only attractive force in such IPNs [138,139], they swell as temperature is increased (compare with the LCST materials above). IPNs have been shown to be effective in pulsatile 'on–off' delivery [42]. The potential effect of hydrogen-bonding proteins loaded in IPNs on

their swelling behavior has not been reported, however.

The gels considered above are prepared by simultaneous copolymerization and crosslinking of monomers with one or two vinyl groups. Another route for synthesis of responsive gels is to crosslink polymers that possess LCST, either through γ-irradiation, as with poly(vinyl methyl ether) [140] or chemically, as with cellulose esters and divinyl monomers [141]. Gehrke et al. [141,142] examined synthesis and drug release using chemically crosslinked gels from methylcellulose, hydroxypropylmethylcellulose, hydroxypropylcellulose and carboxymethylcellulose, all crosslinked with divinyl sulfone. We developed hydrogels for drug delivery from the same polysaccharides, but crosslinked by safe compounds such as adipic, sebacic, or succinic acid [143,144]. Although all studied polysaccharides possess a LCST, their transition temperatures are generally too high to be used in the human body [122], and therefore the hydrogels were used as pH-triggered, rather than temperature-responsive materials in drug delivery [144].

### *2.5. Limitations*

Despite the extensive research effort during the last 10–15 years, practical applications of crosslinked hydrogels in drug delivery are still to emerge as commercial products. We perceive two basic hurdles: one that originates from material limitations, and the other from the concept of temperature-controlled delivery itself. Changes in temperature that will trigger the drug delivery can be either due to increased body temperature in a disease state, or modulated external temperature (in the form of heat-triggered subdermal implants, etc.) [64]. In any case, the requirement of changing the temperature in parts of the human body presents a substantial limitation for controlled-release drug administration which implies prolongation of the duration of drug delivery [145].

On the other hand, gels that are made by chemical reaction should be pure enough to meet the specifications of the FDA, as vinyl monomers and crosslinkers are highly toxic, carcinogenic or teratogenic. If gel formation occurs with the solute to be loaded (such as protein, in many circumstances), then purification of the pre-loaded gels becomes a problem [142]. Moreover, although purified gels are usually benign, the perception that acrylamide-based polymers activate platelets on contact with blood [146], along with the poorly understood metabolism of polyNIPA, make it difficult to win FDA approval. It may be easier to win the approval for responsive gels made from polymers which already have some approved status. Gels from alkyl-substituted cellulose and starch derivatives [142–144] which are on the FDA's 'generally recognized as safe' list may be advantageous in this regard, but their applications in temperature-responsive drug delivery are limited due to the high LCST of the gel backbone polymers, and therefore these gels are beyond the scope of the present review.

## 3. Thermally reversible gels (TRG)

The limitations to the use of permanently crosslinked gels in temperature-controlled drug delivery as discussed above have indicated the need for a change in the 'gel paradigm,' in order to broaden the gel applicability. Namely, if a polymer solution can be found that is a free-flowing liquid at ambient temperature and gels at body temperature, such a system would be easy to administer into a desired body cavity. Moreover, the loading of such a system with a drug could be achieved by simple mixing of the drug with already prepared benign polymer formulation. When gelled, the system would release the drug by mass action law, similar to typical crosslinked gels. Furthermore, if a thermogelling polymer is responsive to some stimulus other than temperature (such as pH), it can be triggered to 'un-gel' at body temperatures, thereby releasing the drug. We undertook the task of introducing the paradigm of thermally reversible gels (TRG) into protein and peptide delivery about two years ago. Let us first consider how it is thermodynamically possible to create TRGs.

### *3.1. TRG: Design by thermodynamics*

Just like a covalently crosslinked network (permanent gel), a reversible gel is characterized by the finite size of the network that is linked into a unit at any moment of time. In a reversible gel, however, since the network connections are dissociable, they

will always be opening, closing, and interchanging. The distinct features of reversible gels were defined by Silberberg [147] to be (i) the structure that is determined by chemical equilibrium between the crosslinking entities, rather than the conditions under which the gel was formed, and (ii) absence of infinite mechanical relaxation times (had the crosslinks been permanent, the relaxation times would have been infinite). Guenet [148] emphasized that, to be stable, the bonding in reversible gels requires some degree of cooperativity which implies that the junction domains are not point-like but extend into space. Interestingly, in his book, Guenet never implies hydrogen bonding [148] but considers only van der Waals interactions. However, numerous examples of reversible gels are found in the biological world where cooperative hydrogen bonding between macromolecules enjoys lifetimes much longer than in water–water associations. The cooperativity effect is reflected in the lowered energy barrier to a hydrogen bond being formed when the neighboring group has been hydrogen bonded already. The free energy of the cooperative hydrogen bonded system is proportional to the square of hydrogen bonding density. Transitions of α-helix to β-sheet structures in proteins and polysaccharides and strand pairing in nucleic acids are among phenomena attributed to cooperative hydrogen bonding. Temperature-reversible gelation can be caused by a random coil–helix transition where on lowering the temperature the random coiled biopolymer chains form double helices that later aggregate and form physical junctions of the gel (Fig. 4) [148].

Examples of aqueous synthetic reversible gels that are hydrogen-bonded systems are poly(vinyl alcohol) crosslinked by poly(ethylene oxide), boric acid and other hydrogen-bonding agents, and systems held together by the crystalline sequences found in syndiotactic poly(methacrylic acid), poly(vinyl alcohol) and other polymers [147–153]. All these systems, however, show behavior opposite to that needed to enable the aforementioned TRG concept in drug delivery: they liquefy when heated. The reason for that is the 'melting' of the ordered crystallites [148] due to the sharp decrease of the lifetimes of hydrogen bonds with increased temperature. Thus it is only hydrophobic interactions that can create crosslinks (and thereby gel the polymer solution)

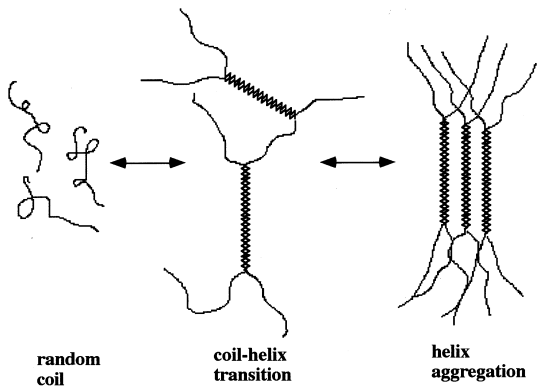



Fig. 4. Formation of physical junctions in the gel due to coil–helix transitions. Adopted, with changes, from Ref. [148].

with the increase in temperature, in a manner similar to the appearance of polymer-rich phases above LCST (see above). An explanation of the conditions of formation of a TRG is given in Ref. [147].

Let us imagine a polymer chain that consists of different segments (blocks), such that upon dissolution in water not only segment–segment contacts exist, but also segment–solvent and solvent–solvent contacts. The energy change ($\Delta G$) per contact when we replace two segment–solvent contacts ($E_{12}$) with a segment–segment ($E_{11}$) and a solvent–solvent ($E_{22}$) contact is [147]:

$$\Delta G = E_{11} + E_{22} - 2E_{12} \quad (1)$$

The situation with the status of a polymeric system is determined by the Flory–Huggins parameter ($\chi$) which is related to $\Delta G$ [84]

$$\chi = \text{const.} \cdot (-\Delta G/K_B T) \quad (2)$$

where $K_B$ is the Boltzmann constant and $T$ is absolute temperature.

In the case of good solubility, $\Delta G < 0$ and $\chi < \frac{1}{2}$. Phase separation starts to occur when

$$\chi \geq 1/2[1/(1 + N^{1/2})] \quad (3)$$

where $N$ is the size of the polymer chain.

The strong segment–segment contacts (which are needed to form associative crosslinks) require negative $E_{11}$ values that are large in absolute terms (because $\Delta G$ must be negative). These large values, however, lead to a very large $\chi > 1/2$, which in turn

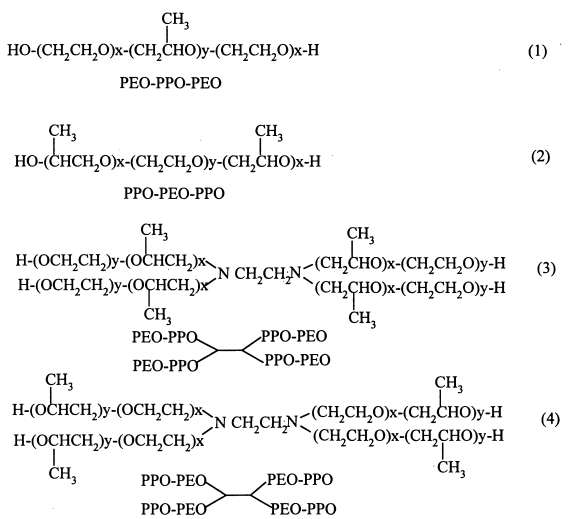
makes the polymer insoluble. One can see conflicting requirements here. The solution is formulated by Silberberg [147] as follows. In order for a polymeric system to form a reversible gel, it has to be a copolymer. A segment that interacts very strongly with itself will form temporary crosslinks, whereas the remainder of the chain, composed of a soluble segment, will permit the system to stay in the solution.

The above theory presents a simple strategy for designing a TRG [154]. Indeed, let us consider a polymeric chain where the hydrophobic, self-aggregating segment is minor, whereas the hydrophilic chain is very long. In this system, despite the large $E_{11}$ due to the minor segment, $E_{12}$ (because of interactions between water and the large hydrophilic segment) will overwhelm the $E_{11}$, thus rendering small $\chi < 1/2$ of the entire system. No major phase separation in the system will be observed, but the number of aggregates (crosslinks) will be small, so the formed gel will be somewhat weak. By varying the lengths and ratios of hydrophilic and hydrophobic segments one can design a TRG with the desired properties. We believe that bonding in TRG should obey the same requirement of cooperativity that holds for reversible gels in general [148]. This implies that the hydrophobic junction domains are to extend into space to yield a reasonably strong gel. Hence, hydrophilic and hydrophobic segments in TRG cannot be of single link size; in other words, TRG should comprise a block- or graft-copolymer of segments of different hydrophobicity, rather than a random copolymer. Random copolymers of a strongly associating component that has LCST and hydrophilic components might be an exception from this notion [155], but there phase separation phenomena would be abundant.

### *3.2. TRG: Emerging materials*

The first publications on the applicability of TRG-like materials in protein and peptide topical delivery appeared about 15 years ago [156,157], so this concept is still relatively young. However, significant advances in the mechanistic and functional understanding of thermogelling materials have already been followed by the development of drug delivery systems. Although Hoffman and co-workers proposed graft- and block-copolymers of poly(*N*-isopropylacrylamide) and ionizable components, such as poly(acrylic acid), as reverse-gelling materials for drug delivery [158–162], the use of poly(*N*-isopropylacrylamide) has serious limitations from the standpoint of difficulty to obtain FDA approval discussed above. Therefore, the vast majority of the drug delivery TRG systems use block-copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) which represent the only up-to-date thermoviscosifying materials approved by FDA and EPA as direct and indirect food additives, pharmaceutical ingredients and agricultural products [163]. The structures of the most frequently used block-copolymers are given in Scheme 1. Copolymers 1, 2, 3, and 4 have trade names Pluronic®, Pluronic® R, Tetronic®, and Tetronic® R, respectively.

Triblock PEO–PPO–PEO copolymers (Pluronics, or Poloxamers) are available in a variety of lengths and are of particular interest to us, as their thermoviscosification and gelation have been extensively studied [164–166]. The surfactant properties of Pluronics, and especially their micelle-forming capability [164,167], have resulted in their use in medical applications. These include drug delivery using the 'microcontainer' concept pursued by Kabanov and co-workers [168–173]. According to this concept, drugs are incorporated into Pluronic micelles which




Scheme 1. Structure of PPO–PEO block-copolymers used in drug delivery.

enhance drug penetration through lung tissues and even through the blood–brain barrier, due to the altered partitioning properties of the solubilized drugs. Interestingly, some multidrug-resistant carcinoma cells showed hypersensitivity to Pluronics, suggesting that the latter can enhance the activity of antineoplastic agents against tumors [171–176]. Yet another attractive property of Pluronics, their resistance to protein adsorption [177–179], allows the creation of biocompatible surfaces in devices for blood contact, membranes for blood purification, and contact lenses [177], as well as conditioning of micro- and nano-spheres for drug delivery [180]. Lowered or suppressed interactions of Pluronics with proteins like immunoglobulins which identify drug vehicles as foreign, prevent the adhesion of drug vehicles onto the surfaces of phagocytes, thereby preventing clearance of the vehicle allowing them to circulate for a long time [181]. In the present work, we review the gelation properties of Pluronics as they pertain to the TRG concept in drug delivery [182–189].

The properties of some Pluronic copolymers frequently used in drug delivery studies are collected in Table 1.

It can be seen that the cloud point (corresponding to LCST) varies from 19°C for the 'hydrophobic' L122 copolymer with low PEO content and low HLB, to above 100°C for Pluronics with high PEO content. Pluronics with LCST as low as 10°C were reported [164]. It is significant to note that although most of the Pluronics listed in Table 1 have a LCST well above normal body temperature, they do exhibit gelation at body temperature in concentrated solutions [164,166,194]. Notably, Hoffman and Chen [158], suggesting that gelation of Pluronics is analogous to LCST phenomenon in polyNIPA solutions [37,159], limited their study to the copolymers that have LCST ranging from about 20 to 40°C, and therefore tested only polyNIPA copolymers and graft-copolymers of Pluronics L122, L61, L81, and L92 with LCST of 19, 24, 20 and 26°C, respectively. Let us consider the actual mechanism of gelation in Pluronic solutions, in light of the general considerations of formation of a reversible gel given by Silberberg [147].

The formation of micelles in aqueous Pluronic solutions has been proven with a wide range of experimental techniques [164,165,195–198]. At higher concentrations and/or elevated temperatures, these micelles associate and form various lyotropic liquid crystalline phases [194,199] (Fig. 5).

The regions of stability of the ordered nanostructures of Pluronics, and especially cubic three-dimensional lattices ('gels'), move toward lower concentrations at higher temperatures [164,196,200,201]. Thus a Pluronic solution may be designed that gels at body temperature by forming a liquid crystalline phase due to increasing intermicellar interactions. At still higher temperatures the gel melts again. The PEO chains in the micellar mantle were shown to strongly interpenetrate [215,216]. The gelation onset and temperature and the thermal stability range of the gel increase with increasing length of the PEO block. Recently, it has been found [194] that the formation of liquid crystalline phases in Pluronics

Table 1
Properties of the Pluronic PEO–PPO–PEO copolymers [164,190,192,193]

| Copolymer[a] | Composition | Average MW | $M_{PPO}$ | PEO (wt%) | CP[b] (°C) | HLB[c] |
|---|---|---|---|---|---|---|
| L64 | $EO_{13}PO_{30}EO_{13}$ | 2900 | 1740 | 50 | 58 | 12–18 |
| F68 | $EO_{76}PO_{29}EO_{76}$ | 8400 | 1680 | 80 | >100 | >24 |
| F88 | $EO_{103}PO_{39}EO_{103}$ | 11 400 | 2280 | 80 | >100 | >24 |
| P103 | $EO_{17}PO_{60}EO_{17}$ | 4950 | 3465 | 30 | 86 | 7–12 |
| P104 | $EO_{27}PO_{61}EO_{27}$ | 5900 | 3540 | 40 | 81 | 12–18 |
| P105 | $EO_{37}PO_{56}EO_{37}$ | 6500 | 3250 | 50 | 91 | 12–18 |
| F108 | $EO_{132}PO_{50}EO_{132}$ | 14 600 | 2920 | 80 | >100 | >24 |
| F127 | $EO_{100}PO_{65}EO_{100}$ | 12 600 | 3780 | 70 | >100 | 18–23 |
| L122 | $EO_{12}PO_{67}EO_{12}$ | 5000 | 3600 | 20 | 19 | 1–7 |

[a]L, F and P indicate liquid, flakes, and paste, respectively.
[b]Cloud point in aqueous 1 wt% solution.
[c]Hydrophilic–lipophilic balance.

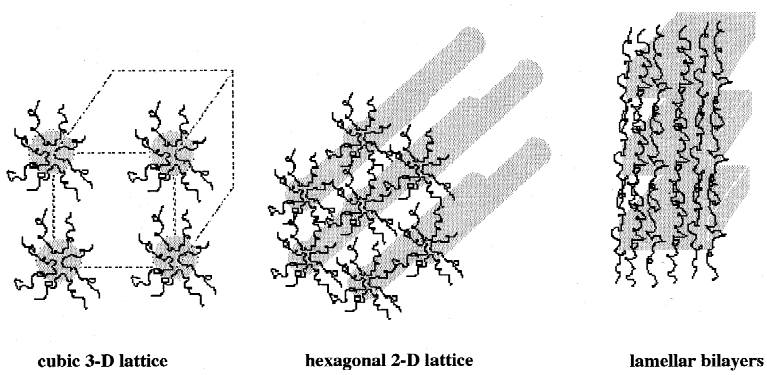



Fig. 5. Lyotropic liquid crystalline structures in concentrated Pluronic solutions. Adopted, with changes, from Ref. [166].

occurs regardless of LCST; it is only the particular structure of the liquid crystal that is related to LCST. In line with the considerations of the TRG discussed above (Eqs. (1)–(3)), it has been established [191,193,194] that as the molecular weight of Pluronics becomes sufficiently high to form micellar aggregates, the shape and packing symmetry of the ordered structures (reflecting stability of gels) depend on the ratio of lengths of the hydrophilic and hydrophobic segments.

### *3.3. TRG: Drug delivery*

The liquid crystalline nanostructure yields a thermoreversible gel capable of solubilizing drugs within the micellar interior. Along with low toxicity, mucomimetic properties, and optical clarity, this provides additional convenience for pharmaceutical formulations applied in drug delivery, especially in the ophthalmic area [202–214]. Since F127 has been reported to be the least toxic of the commercially available Pluronics [199], it has been used most extensively in drug delivery studies. Gilbert et al. [210,211] demonstrated the possibility of controlled release of low-molecular-weight compounds from the Pluronic F127 gels. Interestingly, the more lipophilic drugs were added into the gels the greater the decrease of the temperature of their sol–gel transition. Addition of PEO homopolymers generally increased the gelation temperature, depending on the length and concentration of the added PEO. However, PEO added at increasing concentration caused 'melting' of the gels, and at very high PEO concentration phase separation occurred [217,218]. The 'melting' of gels was observed with addition of some polyelectrolytes [217] and surfactants [219,220]. PPO as an additive tended to increase the stability region of the gel, depending on the length of the homopolymer [221]. The stability region of the gel phases strongly depends on the presence of inorganic salts, due to the change of micellar size resulting from dehydration of the micelles [217,222,223]. Addition of sodium chloride to concentrated solutions of Pluronic F127 increased the slope of the viscosity versus temperature relationship and reduced the diffusion coefficient of a polypeptide, atrial natriuretic factor, in the Pluronic gels [223]. These studies provide a fundamental understanding of the TRG pharmaceutical formulations in drug delivery.

Release profiles of phenolsulfophthalein from Pluronic F127 gel formulations were analyzed by Guzman et al. [207]. It was found that the in vitro release rates were inversely proportional to the Pluronic concentrations and a zero-order release rate was observed. Plasma levels of phenolsulfophthalein administered subcutaneously into rats reached a plateau within few minutes that lasted for 8–9 h. The data showed a good fit to a zero-order input and first-order output in the two-compartment pharmacokinetic model, indicating that it was the release from the gel that controlled the overall drug absorption process.

Examples of controlled protein release using Pluronic formulations are given by Langer and his group [208,209]. A biodegradable polymer, poly(L-lactic acid), was blended with Pluronics and fluores-

cein-labeled bovine serum albumin, and the blend films were tested for the protein release under batch conditions. It was observed that the protein dumping at the initial stage of release ('burst' effect) was lowered in the presence of Pluronics. The ability of the Pluronics to delay protein release (lower the 'burst' effect) and prolong the release period increased in the range of Pluronics F108<L101, which the authors [209] explained by the fact that the more hydrophobic Pluronic leached from the blend films more slowly than the more hydrophilic ones. It was suggested that the diffusion coefficient of the large protein in the gel structure of the Pluronic is reduced when compared to that in the aqueous phase, although the question remained of whether the liquid crystalline gel phase stays unperturbed when Pluronic is blended with a large amount of Biodegradable polymer, poly(L-lactic acid) [209].

In a series of papers by Johnston and co-workers [224–228] Pluronic F127 gels were used for the sustained delivery of interleukin-2 following intraperitoneal injection in mice and rats, and to achieve sustained release of urease from a gel matrix. Interestingly, Pluronic environment was shown to enhance stability of both proteins in aqueous solutions. Banga et al [229,230] studied sustained release and stability of human growth hormone, a 22 kDa protein, using Pluronic gels and observed zero order release profile at 37°C and controlled release in vivo following intramuscular and subcutaneous injections in rats. Pluronic gels, once again, were shown to enhance protein stability [229].

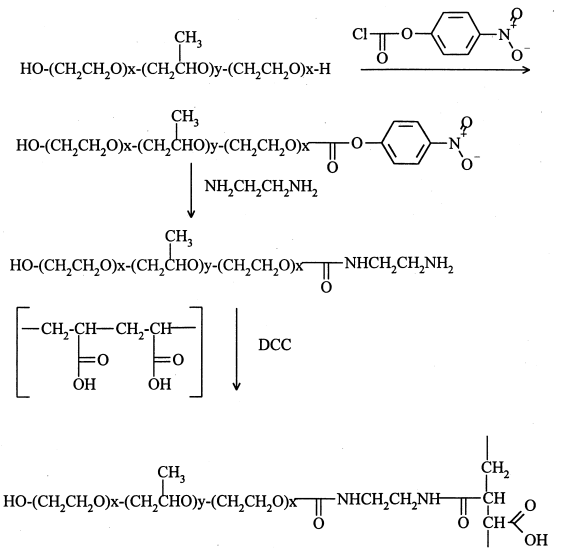
### *3.4. TRG: Novel 'intelligent' copolymers*

The formation of a TRG using safe materials such as Pluronics provides flexibility for a pharmaceutical formulation and in general answers the call for a free-flowing liquid at ambient temperature that gels at body temperature. However, the very nature of gelation caused by formation of densely packed liquid crystalline phases in concentrated Pluronic solutions limits the applicability of Pluronics in drug delivery. Indeed, the lowest concentration of, for instance, Pluronic F127 that forms gel at body temperature in aqueous solutions was reported to be 16 wt% [231]. However, application of concentrated polymer solutions in drug delivery may be disadvantageous as it changes the osmolality of the formulation, kinetics of gelation, and causes discomfort in ophthalmic applications due to vision blurring and crusting [186]. Joshi et al. [186] tried to address this problem by lowering the necessary amount of thermogelling polymer by blending it with a pH-sensitive reversibly gelling polymer. The latter could be a polymer that contains carboxyl groups which can change ionization degree in response to the pH and ion composition of tear fluid or another bodily fluid. This fine concept, however, suffers from the drawback of insurmountable phase separation in physical blends.

Given these premises, Hoffman et al. [37,158–162] and the present authors [154,232–239] developed a new generation of 'intelligent' copolymers of thermogelling surfactants and pH-responsive polymers containing ionizable carboxylic groups. Chemical bonding between temperature-and pH-sensitive components provides the necessary stability to the copolymer absent in a physical blend. The presence of safe COOH-containing segments imparts the attractive advantage of prolonging residence time of topical formulations due to adherence to mucosal surfaces [37,158].

In agreement with the notion of the impact of ionization on a gel's LCST [30], it was found that random copolymers of the temperature- and pH-sensitive monomers lose their temperature-sensitivity at body temperatures when the levels of the pH-sensitive component are high enough to obtain a sufficiently bioadhesive material [37,158]. It therefore appears to be necessary to obtain a graft copolymer structure because it (i) combines bioadhesive and hydrophobic properties in a single molecule, (ii) retains TRG behavior over a wide pH range, and (iii) does not permit physical separation [159]. The synthetic route with which Hoffman and Chen [158] approached this problem is depicted in Scheme 2.

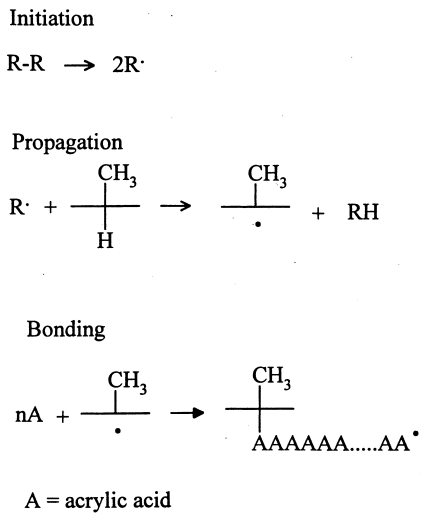
First, Pluronic was activated by derivatization with 4-nitrophenyl formate in the presence of triethylamine. After purification, the intermediate was reacted with diaminoethylene to yield an amino-terminated Pluronic which was conjugated to poly(acrylic acid) via an amide bond applying dicyclohexylcarbodiimide (DCC) as a coupling agent. The resulting Pluronic-*g*-poly(acrylic acid) conjugates were shown to viscosify even in diluted




Scheme 2. Synthesis of Pluronic–poly(acrylic acid) graft-copolymer [158].

(1–3 wt%) aqueous solutions when warmed to body temperatures at physiological pH. The structure of the conjugates suggested implies that only one terminus of Pluronic would have an amino group capable of conjugating with poly(acrylic acid). Synthesis of monoamino-terminated Pluronic may be a problem on an industrial scale. Applying Pluronic derivative aminated on both ends would lead to a permanent crosslinking of the poly(acrylic acid) chains with the Pluronic segments by urethane bonding. Another hurdle is the three-step procedure itself with several intermediate steps of purification of the sub-products.

In our one-step scaled up synthetic procedure the property of Pluronics to form macromolecular radicals by hydrogen abstraction from methyne and methylene groups and thus undergo polymeranalogous transformations [240] was explored. Copolymerization of Pluronic involves attack on the secondary or tertiary carbons by free radicals resulting from a radical polymerization initiator, such as peroxides, persulfates, azo-compounds, etc. Generation of a macromolecular Pluronic-radical in the presence of vinyl monomers such as acrylic acid will lead to the bonding of a growing poly(acrylic acid) chain onto the polyether backbone, as depicted in Scheme 3 [154].




Scheme 3. Synthesis of Smart Hydrogel™ [154].

The reactions in Scheme 3 are exemplified by propylene oxide residue, but they are also applicable to ethylene oxide. The free-radical polymerization is a scaleable, potentially continuous process yielding graft-copolymers which were optimized to give an essentially monomer-free product, poly(oxyethylene-*b*-oxypropylene-*b*-oxyethylene)-*g*-poly(acrylic acid) (trade name Smart Hydrogel™).

A striking feature of Smart Hydrogel™ aqueous solutions is their ability to thermogel at body temperatures, without phase separation, at low polymer concentrations when neither parent Pluronic nor a physical blend of Pluronic and poly(acrylic acid) show any signs of viscosification [234]. At temperatures of 20°C or below the viscosity of 1–5 wt% aqueous solutions of Smart Hydrogel™ (weight ratio of poly(acrylic acid) to Pluronic F127 in the graft-copolymer is ca. 1:1, pH 7.0) does not vary significantly. However, at higher temperatures, a rapid $10–10^3$-fold increase in viscosity occurs over a range of several degrees [233]. Finally, a plateau is reached where the viscosity does not change with temperature. At body temperatures, when the Smart Hydrogel™ solution is in its gel state, the storage modulus is much greater than the loss modulus, and both moduli are almost independent of the frequency of oscillatory shear. This is a behavior typical of a viscoelastic solid. Smart Hydrogel™ exhibits a substantial thermothinning effect when gelled, a

property potentially advantageous for drug delivery when formulation can be delivered if sufficiently high shear is available [233].

We believe the gelation of the Smart Hydrogel™ solutions may be phenomenologically analogous to the formation of gel phases in concentrated Pluronic solutions. Dynamic light scattering experiments show increase of the intensity of the scattered light at temperatures almost coincidental with the gelation of Smart Hydrogel™ [235] (Fig. 6).

These data indicate a growing number of aggregates of the graft-copolymer molecules. In line with the observation that gelation in concentrated Pluronic solutions occurs regardless of LCST [194] the thermothickening behavior of the Smart Hydrogel™ solutions was independent of whether or not cloudiness was observed. For instance, when a 1 wt% aqueous solution of copolymer of Pluronic F127 and poly(acrylic acid) (weight ratio ca. 1:1) was heated, an almost $10^2$-fold increase in viscosity was observed at about 27°C. No cloudiness was observed in this system in the range 0–100°C. In contrast, a very marked cloudiness was observed (onset of phase separation at 33°C) under identical conditions in the system based on Pluronic L-92:poly(acrylic acid) (1:1). The cloud point was followed by gelation. These differences must be governed by the differences of the hydrophilicity in these two Smart Hydrogel™ systems (compare the EO contents of Pluronic F127 and L92, being 70 and 20%, respectively). It was demonstrated therefore that phase separation of the entire Smart Hydrogel™ system is not required for, and may be even unrelated to, thermogelation, given the large $E_{11}$ of the hydrophobic segment and yet the large $E_{12}$ of the entire copolymer (see Eqs. (1)–(3)). The importance of this observation is evident for ophthalmic drug delivery, where the formulation, although gelled, remains waterlike-clear.

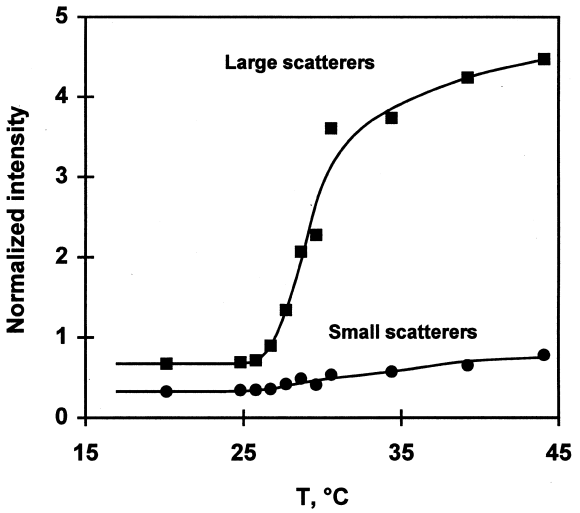



Fig. 6. Light scattering intensities of small particles and their larger aggregates in Smart Hydrogel™ solution [235].

It is hard to assume the appearance of tightly packed cubic liquid crystalline phases in Smart Hydrogel™ because the formation of such phases requires a material of low polydispersity that can form highly symmetrical structures of long-range order. Studies are currently underway to elucidate the nature of structural changes in Smart Hydrogel™ upon gelation.

Since both components of Smart Hydrogel™, Pluronic and poly(acrylic acid), have an approved regulatory status [163,241], it can be expected that their graft-copolymers would be safe. Indeed, our animal toxicological study showed the non-toxic nature of Smart Hydrogel™ (Table 2).

In addition to lack of irritancy, it is important to show that the material stays in place for a prolonged time when applied to a mucous tissue as a vehicle for

Table 2
Toxicology of Smart Hydrogel™ thermogelling materials

| Test | Subject | Result |
|---|---|---|
| Skin sensitization | Guinea pigs | No skin sensitization |
| Acute oral | Rats | No acute toxicity |
| Acute dermal | Rabbits | No acute toxicity |
| Primary skin irritation | Rabbits | No irritancy |
| Primary eye irritation | Rabbits | No irritancy |
| Microsome mutagenesis assay | *Salmonella typhimirium* | Not mutagenic |

topical drug delivery. The bioadhesive force between pig gastric mucin and poly(oxyethylene-*b*-oxypropylene-*b*-oxyethylene)-*g*-poly(acrylic acid) copolymers and Pluronic F127 was measured by the rheological method of Hassan and Gallo [242] and compared to those of a mucoadhesive material, such as poly(acrylic acid). While no substantial bioadhesion of Pluronic F127 was found, the bioadhesive bond strengths of high-molecular-weight poly(acrylic acid) and Smart Hydrogel™ were similar. The bioadhesive force was observed to be higher at pH 5.4 than 2.0 (Fig. 7).

This result corresponded well with the data on interactions of poly(acrylic acid) and Smart Hydrogel™ with rabbit vaginal tissue. It has also been shown that Smart Hydrogel™ can effectively adhere to the human oesophageal mucosa [237]. A solution of Smart Hydrogel™ radiolabelled with $^{99m}$Tc-diethylenetriaminepentaacetic acid was administered orally and scintigrafic dynamic imaging was performed (Fig. 8).

Biphase clearance of Smart Hydrogel™ was observed. First, a viscous solution comprising about 65% of the administered dose quickly passed into the stomach, and then about 15% of the dose displayed prolonged retention in the oesophagus. This gelled coating of the oesophagus may be advantageous in gastro-oesophageal reflux disease, whereas the prolonged residence time may be beneficial in reducing inflammatory conditions. The concept of coating the damaged oesophagus with a material which is resistant to acid damage has not, to our knowledge, been explored. Employing a mucoadhesive hydrogel to 'bandage' the oesophagus, and thus protect the mucosa from the harmful gastric contents, may be useful both as a prophylactic measure and as a platform for drug delivery to the damaged oesophagus [237].

Thermogelation, bioadhesive properties, and the ability of Smart Hydrogel™ to solubilize hydrophobic drugs in aqueous media make it an effective vehicle for vaginal delivery of steroid hormones [239]. In vitro release studies demonstrated two modes of transport of lipophilic drugs from the gelled systems: the drug that was not incorporated into the aggregates of poly(oxyethylene-*b*-oxypropylene-*b*-oxyethylene)-*g*-poly(acrylic acid) (Pluronic F127:poly(acrylic acid) weight ratio ca. 1:1) was released by Fickian diffusion, whereas erosion of the Smart Hydrogel™ by hydrodynamic flow allowed for the transport of the drug not associated with the copolymer aggregates. Importantly, the initial rate of hormone release increased with either decreasing total polymer concentration in the formulation or decreasing temperature. The rate of transport was shown to be reciprocally proportional to the macroscopic viscosity of the Smart Hydrogel™. In animal in vivo studies formulations were administered as a free-flowing system providing coverage of the vaginal cavity. Gelation of the system helped to prevent the leak-back. The pharmacokinetics of estradiol release from Smart Hydrogel™ in sheep showed about a 5-fold higher bioavailability when compared to commercial systems, such as Estrace™ (Bristol-Myers Squibb) and Premarin™ (American Home Products) [239].

In vitro protein release studies demonstrated results similar to hormonal release studies [243]. The viscosity of the Smart Hydrogel™ solutions was reciprocally proportional to the initial release rate

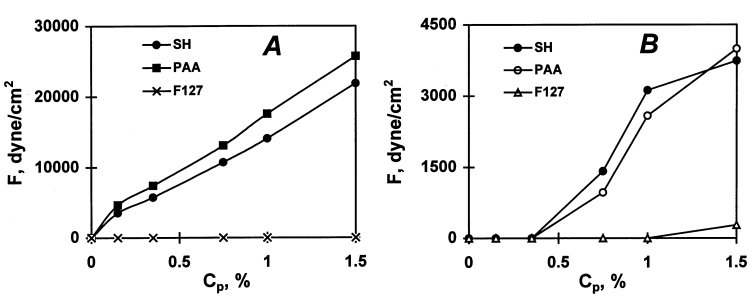



Fig. 7. Bioadhesive force ($F$) as a function of concentration of bioadhesive polymer ($C_p$). $T = 25$°C, shear rate 24 $s^{-1}$, mucin concentration 15%. (A) pH 5.4; (B) pH 2.0.

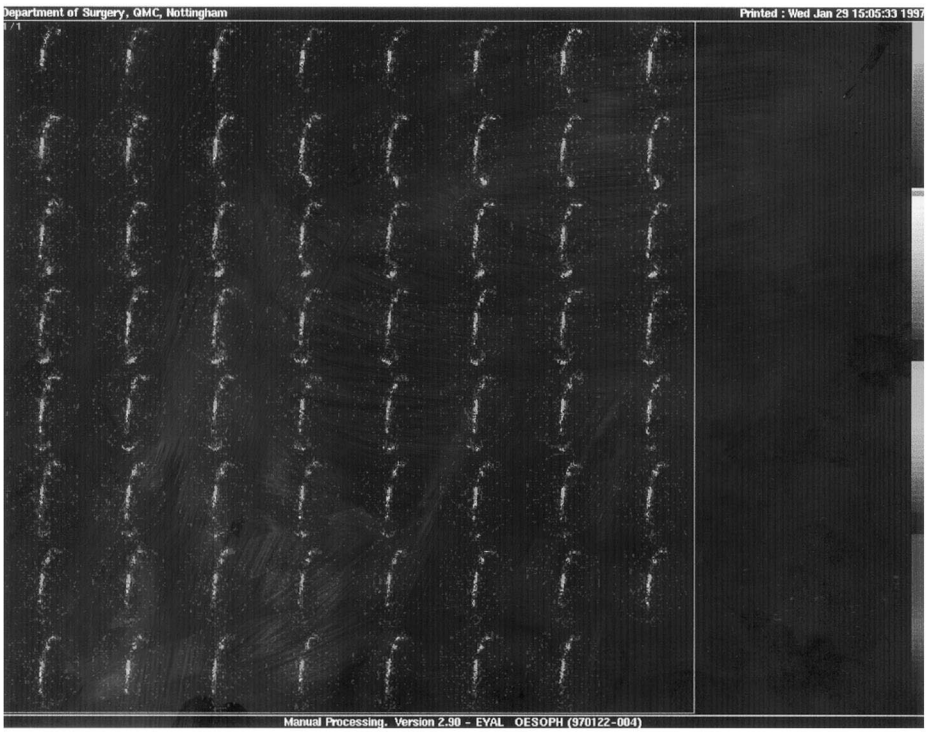



Fig. 8. Scintigraphic image of the $^{99m}$Tc-DTPA labelled Smart Hydrogel™ formulation in the oesophagus 10 min after administration. Oesophagus appears as an oblong, mostly red spot in the middle of the image. Also shown are oropharynx and stomach (smaller spots above and below oesophagus, respectively). Concentration of the radiolabel decreases in the sequence red>yellow>green>blue. The background (no counts) is given in black. The photograph is the courtesy of Drs. Neena Washington (Department of Surgery, Queen's Medical Centre, Nottingham, UK) and Clive Wilson (Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow, UK).

and therefore regulation of the viscosity afforded control of the release rate. The ability of the Smart Hydrogel™ to lower the 'burst' effect and prolong the release period of proteins, such as $Zn^{2+}$-insulin, hemoglobin and lysozyme, increased as the solution gelled at body temperatures. The protein and peptide release rates could be varied depending on the total polymer concentration (Fig. 9). Initial dumping of

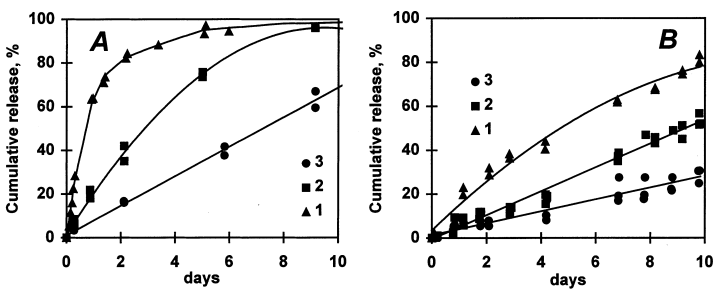



Fig. 9. Kinetics of LHRH (A) and human recombinant insulin (B) release from isotonic buffer solution (1), 1% solution of Smart Hydrogel™ (2) and 5% solution of Smart Hydrogel™ (3) through dialysis membrane (molecular weight cut-off 100 000) into aqueous isotonic solution. $T=37$°C, pH 7.0. Kinetics corrected for the membrane resistance are described in Ref. [244].

LHRH and insulin into the receiver solution was lowered in the presence of Smart Hydrogel™, and notably, at 5% polymer concentration in the Smart Hydrogel™ formulation, a zero-order peptide release was observed (Fig. 9).

Preservation of the enzymatic activity of lysozyme loaded into and released from the Smart Hydrogel™ through a porous membrane into phosphate-buffered saline indicated the absence of deleterious processes in the enzyme upon contact with the Smart Hydrogel™ [243]. It was suggested [243] that proteins remain in their native state when released from Smart Hydrogel™ formulations into an aqueous medium. This was further supported by the electronic absorption spectra of human hemoglobin transferred through the membrane from the Smart Hydrogel™ into an aqueous receiver solution. The maxima of the Soret band, as well as β and α bands due to the $\pi \rightarrow \pi^*$ electronic transitions in heme, corresponded to the native state of oxidized human hemoglobin. Along with the use of Pluronic-based formulations [208,209], the above studies are the first examples of the application of the TRG concept in protein and peptide delivery [244,245].

## 4. Concluding remarks

The majority of current drug formulations represent lyophobic colloids, such as suspensions, emulsions, powders, crosslinked gels, etc. These colloids are thermodynamically unstable, i.e. they require additional energy to be applied for their dispersion. Recently, however, a new trend toward application of lyophilic colloids in drug delivery has emerged [246]. Lyophilic colloids spontaneously assemble from macroscopic phases and are thermodynamically stable. It has been stated [246] that this is a major trend in modern pharmaceutics due to the increasing need for multifunctional delivery systems which can carry a drug into its target site in the body, transport it into the cell and even direct it into the desired intracellular compartment. We believe that the property of Pluronic copolymers and Smart Hydrogel™ to associate spontaneously while carrying a drug embodies such a lyophilic multifunctional delivery system. Beyond the ability to gel and thus create an effective depot for the drug, Pluronics and perhaps Smart Hydrogel™ and/or their mixtures, when in contact with living cells, may alter endocytosis [246–248], thereby changing the mode of drug transport into the cell. The responsiveness of block-copolymers to both temperature and pH [154,158] not only provides a convenient way to administer a drug to the treatment area, but may perhaps provide a means of realization of advanced concepts based on application of polymer–protein conjugates such as control of a protein's ligand binding affinity [249] or an intelligent delivery system that is inert with respect to non-target cells [246]. In this regard, we envision serious limitations for lyophobic colloids such as permanently crosslinked gels. The trend toward the TRG concept using 'traditional' materials possessing well-defined LCST has been manifested by application of uncrosslinked copolymers of poly(*N*-isopropylacrylamide) in protein and peptide drug delivery [132,133].

## Note added in proof

In a recent article Kim and co-workers forwarded an interesting approach toward the use of a TRG-like system in subcutaneous delivery [250]. Diblock and triblock copolymers of poly(ethylene oxide) and poly(L-lactic acid) (PLLA) were shown to undergo sol-gel transition in a range of temperatures which could be manipulated by varying the length of the hydrophobic, biodegradable segment (PLLA). The drug delivery concept comprises the application of a solution at around 45°C which becomes a viscoelastic gel at 37°C after subcutaneous injection into the body.

## Acknowledgements

The authors are grateful to Dr. E.C. Lupton, Jr., Dr. Michal Orkisz and Thomas H.E. Mendum for practical help. The in vivo study of the oesophageal retention of Smart Hydrogel™ performed by Drs. C.G. Wilson, P. Gilchrist and A.M. Potts (University of Strathclyde) and Drs. N. Washington and S. Jackson (Queen's Medical Centre, Nottingham) is gratefully acknowledged. We are indebted to Prof. Toyoichi Tanaka (Massachusetts Institute of Tech-

nology), Prof. Allan S. Hoffman (University of Washington), Prof. Stevin H. Gehrke (University of Cincinnati), and Prof. T. Alan Hatton (Massachusetts Institute of Technology) for generous advice.

## References

[1] V.H. Lee, Changing needs in drug delivery in the era of peptide and protein drugs, in: V.H.L. Lee (Ed.), Peptide and Protein Drug Delivery, Marcel Dekker, New York, 1991, pp. 1–56.

[2] M.M. Struck, Biopharmaceutical R&D success rates and development times: A new analysis provides benchmarks for the future, Bio/Technol. 12 (1994) 674–677.

[3] J.E. Talmadse, The pharmaceutics and delivery of therapeutic polypeptides and proteins, Adv. Drug. Deliv. Rev. 10 (1993) 247–299.

[4] R. Langer, J. Folkman, Polymers for the sustained release of proteins and other macromolecules, Nature 263 (1976) 797–800.

[5] M.J. Alonso, R.K. Gupta, C. Min, G.R. Siber, R. Langer, Microspheres as controlled-release tetanus toxoid delivery systems, Vaccine 12 (1994) 299–306.

[6] C. Thies, Formation of degradable drug-loaded microparticles by in-liquid drying processes, in: M. Donbrow, Ed., Microcapsules and Nanoparticles in Medicine and Pharmacy, CRC Press, London, 1992, pp. 47-71.

[7] E. Ron, T. Turek, E. Mathiowitz, M. Chasin, M. Hageman, R. Langer, Controlled release of polypeptides from polyanhydrides, Proc. Natl. Acad. Sci. USA 90 (1993) 4176–4180.

[8] R. Langer, New methods of drug delivery, Science 249 (1990) 1527–1533.

[9] Y. Ogawa, H. Okada, M. Yamamoto, T. Shimamoto, In vivo release profiles of leuprolide acetate from microcapsules prepared with polylactic acid or co-poly(lactic/glycolic acids) and in vivo degradation of these polymers, Chem. Pharm. Bull. 36 (1988) 2576–2581.

[10] V.H.L. Lee, A. Yamamoto, Penetration and enzymatic barriers to peptide and protein absorption, Adv. Drug. Deliv. Rev. 4 (1990) 171–207.

[11] M.C. Manning, K. Patel, R.T. Borchardt, Stability of protein pharmaceuticals, Pharm. Res. 6 (1989) 903–918.

[12] W.D. Lougheed, H. Woulfe-Flanagan, J.R. Clement, A.M. Abisser, Insulin aggregation in artificial delivery systems, Diabetologia 19 (1980) 1–9.

[13] V. Sluzky, J.A. Tamada, A.M. Klibanov, R. Langer, Kinetics of insulin aggregation in aqueous solutions upon agitation in the presence of hydrophobic surfaces, Proc. Natl. Acad. Sci. USA 88 (1991) 9377–9381.

[14] S.P. Schwendeman, M. Cardamone, A. Klibanov, R. Langer, M.R. Brandon, Stability of proteins and their delivery from biodegradable polymer microspheres, in: S. Cohen, H. Bernstein (Eds.), Microparticulate Systems for the Delivery of Proteins and Vaccines, Marcel Dekker, New York, 1996, pp. 1–49.

[15] A.G. de Boer (Ed.), Drug Absorption Enhancement. Concepts, Possibilities, Limitations and Trends, Harwood Academic Publishers, Switzerland, 1994, 489 pp.

[16] L.L. Wearley, Recent progress in protein and peptide delivery by noninvasive routes, Crit. Rev. Ther. Drug Carrier Syst. 8 (1991) 331–394.

[17] L. Narawane, V.H.L. Lee, Absorption barriers, in: A.G. de Boer (Ed.), Drug Absorption Enhancement. Concepts, Possibilities, Limitations and Trends, Harwood Academic Publishers, Switzerland, 1994, pp. 1–66.

[18] S. Zalipsky, C. Lee, Use of functionalized poly(ethylene glycol)s for modification of polypeptides, in: J.M. Harris (Ed.), Poly(Ethylene Glycol) Chemistry. Biotechnical and Biomedical Applications, Plenum Press, New York, 1992, pp. 347–370.

[19] N.V. Katre, The conjugation of proteins with polyethylene glycol and other polymers, Adv. Drug Deliv. Rev. 10 (1993) 91–114.

[20] A.S. Harris, I.M. Nilsson, Z.G. Wagner, U. Alkner, Intranasal administration of peptides: Nasal deposition, biological response, and absorption of desmopressin, J. Pharm. Sci. 75 (1986) 1085–1087.

[21] T. Nagai, Y. Nishimoto, N. Nambu, Y. Suzuki, K. Sekine, Powder dosage form of insulin for nasal administration, J. Control. Release 1 (1984) 15–22.

[22] L. Illum, H. Jørgensen, H. Bisgaard, O. Krøgsgaard, N. Rossing, Bioadhesive microspheres as a potential nasal drug delivery system, Int. J. Pharm. 39 (1987) 189–199.

[23] K. Morimoto, K. Morisaka, A. Kamada, Enhancement of nasal absorption of insulin and calcitonin using polyacrylic acid gel, J. Pharm. Pharmacol. 37 (1985) 134–136.

[24] K. Morimoto, H. Akatsuchi, R. Aikawa, M. Morishita, K. Morisaka, Enhanced rectal absorption of [$Asu^{1,7}$]-eel calcitonin in rats using polyacrylic acid aqueous gel base, J. Pharm. Sci. 73 (1984) 1366–1368.

[25] K. Morimoto, T. Takeeda, Y. Nakamoto, K. Morisaka, Effective vaginal absorption of insulin in diabetic rats and rabbits using polyacrylic acid aqueous gel bases, Int. J. Pharm. 12 (1982) 107–111.

[26] J.L. West, J.A. Hubbell, Localized intravascular protein delivery from photopolymerized hydrogels, in: Proceedings of the International Symposium on the Controlled Release of Bioactive Materials, Controlled Release Society, 22, 1995, pp. 17–18.

[27] H. Ghandehari, P. Kopeckova, P.-Y. Yeh, H. Ellens, P.L. Smith, J. Kopecek, Oral colon-specific protein and peptide delivery: Polymer system and permeability characteristics, in: Proceedings of the International Symposium on the Controlled Release of Bioactive Materials, Controlled Release Society, 23, 1996, pp. 59–60.

[28] P.-F. Bai, L.L. Chang, J.-H. Guo, The use of polyacrylic polymers for improving oral absorption of peptide and protein drugs, in: Proceedings of the Seventh International Symposium on Recent Advances in Drug Delivery Systems, Salt Lake City, 1995, pp. 153–154.

[29] Y. Li, T. Tanaka, Study of the universality class of the gel network system, J. Chem. Phys. 90 (1989) 5161–5166.
[30] M. Shibayama, T. Tanaka, Volume phase transition and related phenomena of polymer gels, Adv. Polym. Sci. 109 (1993) 1–62.
[31] K. Almdal, J. Dyre, S. Hvidt, O. Kramer, Towards a phenomenological definition of the term 'gel', Polymer Gels and Networks 1 (1993) 5–17.
[32] S.H. Gehrke, P.I. Lee, Hydrogels for drug delivery systems, in: P. Tyle (Ed.), Specialized Drug Delivery Systems, Marcel Dekker, New York, 1990, Chapter 8.
[33] A.S. Hoffman, Application of thermally reversible polymers and hydrogels in therapeutics and diagnostics, J. Control. Release 6 (1987) 297–305.
[34] A.S. Hoffman, 'Intelligent' polymers in medicine and biotechnology, Artif. Organs 19 (1995) 458–467.
[35] A.S. Hoffman, A. Afrassiabi, L.C. Dong, Thermally reversible hydrogels: II. Delivery and selective removal of substances from aqueous solutions, J. Control. Release 4 (1986) 213–222.
[36] A. Afrassiabi, A.S. Hoffman, L.A. Caldwell, Effect of temperature on the release rate of biomolecules from thermally reversible hydrogels, J. Membr. Sci. 33 (1987) 191–200.
[37] G. Chen, A.S. Hoffman, Graft copolymers that exhibit temperature-induced phase transition over a wide range of pH, Nature 373 (1995) 49–52.
[38] T. Okano, Y.H. Bae, H. Jacobs, S.W. Kim, Thermally on–off switching polymers for drug permeation and release, J. Control. Release 11 (1990) 255–265.
[39] Y.H. Bae, T. Okano, S.W. Kim, 'On–off' control of solute transport, II. Solute release from thermosensitive hydrogels, Pharm. Res. 8 (1991) 624–628.
[40] Y.H. Bae, T. Okano, S.W. Kim, Insulin permeation through thermo-sensitive hydrogels, J. Control. Release 9 (1989) 271–279.
[41] T. Okano, Molecular design of stimuli-responsive hydrogels for temporal controlled drug delivery, in: Proceedings of the International Symposium on the Controlled Release of Bioactive Materials, Controlled Release Society, 22 1995, pp. 111–112.
[42] T. Okano, Molecular design of temperature-responsive polymers as intelligent materials, Adv. Polym. Sci. 110 (1993) 179–200.
[43] N.A. Peppas, S.K. Vakkalanka, C.S. Brazel, Unique swelling-controlled release systems based on T- and pH-sensitive terpolymers for fibrinolytic enzyme delivery, in: Proceedings of the International Symposium on the Controlled Release of Bioactive Materials, Controlled Release Society, 23, 1996, pp. 267–268.
[44] C.S. Brazel, N.A. Peppas, Synthesis and characterization of thermo- and chemo-mechanically responsive poly(*N*-isopropylacrylamide-*co*-methacrylic acid) hydrogels, Macromolecules 28 (1995) 8016–8020.
[45] N.A. Peppas, S. Vakkalanka, C.S. Brazel, A.S. Luttrell, N.K. Mongia, Controlled release systems using swellable random and block copolymers and terpolymers, in: N. Ogata, S.W. Kim, J. Feijen, T. Okano (Eds.), Advanced Biomaterials in Biomedical Engineering and Drug Delivery Systems, Springer, Tokyo, 1996, pp. 3–7.
[46] T. Amiya, Y. Hirokawa, Y. Hirose, Y. Li, T. Tanaka, Reentrant phase transition of *N*-isopropylacrylamide gels in mixed solvents, J. Chem. Phys. 86 (1987) 2375–2379.
[47] M. Annaka, T. Tanaka, Y. Osada, Volume phase transitions of gels in hydrocarbons, Macromolecules 25 (1992) 4826–4827.
[48] P.L. Privalov, S.A. Potekhin, Scanning microcalorimetry in studying temperature-induced changes in proteins, Methods Enzymol. 131 (1986) 4–51.
[49] P.L. Privalov, Stability of proteins, Adv. Protein Chem. 33 (1979) 167–241.
[50] D.W. Urry, Molecular machines: How motion and other functions of living organisms can result from reversible chemical changes, Angew. Chem. Int. Ed. Engl. 32 (1993) 819–841.
[51] L.D. Taylor, L.D. Cerankowski, Preparation of films exhibiting a balanced temperature dependence to permeation by aqueous solutions: A study of lower consolute behavior, J. Polym. Sci., Part A: Polym. Chem. 13 (1975) 2551–2570.
[52] S. Lewin, Displacement of Water and its Control of Biochemical Reactions, Academic Press, New York, 1974, p. 71.
[53] K.-O. Eriksson, Hydrophobic interaction chromatography, in: J.-C. Janson, L. Rydén (Eds.), Protein Purification. Principles, High Resolution Methods, and Applications, VCH, New York, 1989, pp. 207–226.
[54] H.G. Schild, Poly(*N*-isopropylacrylamide): Experiment, theory and application, Prog. Polym. Sci. 17 (1992) 163–249.
[55] S. Fujishige, K. Kubota, I. Ando, Phase transition of aqueous solutions of poly(*N*-isopropylacrylamide) and poly(*N*-isopropylmethacrylamide), J. Phys. Chem. 93 (1989) 3311–3313.
[56] K. Kubota, S. Fujishige, I. Ando, Single-chain transition of poly(*N*-isopropylacrylamide) in water, J. Phys. Chem. 94 (1990) 5154–5158.
[57] H. Inomata, S. Goto, S. Saito, Phase transition of N-substituted acrylamide gels, Macromolecules 23 (1990) 4887–4888.
[58] O. Katsuto, H. Inomata, S. Goto, S. Saito, Thermal analysis of the volume phase transition with *N*-isopropylacrylamide gels, Macromolecules 23 (1990) 283–289.
[59] A. Matsuyama, F. Tanaka, Theory of solvation-induced reentrant coil–globule transition of an isolated polymer chain, J. Chem. Phys. 94 (1991) 781–786.
[60] M.M. Prange, H.H. Hooper, J.M. Prausnitz, Thermodynamics of aqueous systems containing hydrophilic polymers or gels, Am. Inst. Chem. Eng. J. 35 (1989) 803–813.
[61] F.M. Winnik, Fluorescence studies of aqueous solutions of poly(*N*-isopropylacrylamide) below and above their LCST, Macromolecules 23 (1990) 233–242.
[62] A.S. Hoffman, Conventional and environmentally-sensitive hydrogels for medical and industrial uses: A review paper, in: D. DeRossi, K. Kajiwara, Y. Osada, A. Yamauchi (Eds.), Polymer Gels. Fundamentals and Biomedical Applications, Plenum Press, New York, 1991, pp. 289–297.

[63] T.G. Park, A.S. Hoffman, Immobilization of β-galactosidase in thermally reversible hydrogel beads, J. Biomed. Mater. Res. 24 (1990) 21–38.
[64] T. Okano, R. Yoshida, K. Sakai, Y. Sakurai, Thermo-responsive polymeric hydrogels and their application to pulsatile drug release, in: D. DeRossi, K. Kajiwara, Y. Osada, A. Yamauchi (Eds.), Polymer Gels. Fundamentals and Biomedical Applications, Plenum Press, New York, 1991, pp. 299–308.
[65] Y.H. Bae, T. Okano, S.W. Kim, Insulin permeation through thermo-sensitive hydrogels, J. Control. Release 9 (1989) 271–279.
[66] T. Okano, Y.H. Bae, S.W. Kim, Temperature responsive controlled drug delivery, in: J. Kost (Ed.), Pulsed and Self-Regulated Drug Delivery, CRC Press, Boca Raton, 1990, pp. 17–46.
[67] E.L. Cussler, M.R. Stokar, J.E. Varberg, Gels as size selective extraction solvents, Am. Inst. Chem. Eng. J. 30 (1984) 578–582.
[68] E.L. Cussler, A temperature-sensitive method of size-selective extraction from solutions, U.S. Pat. 4,828,701, 1989.
[69] W.K. Idol, J.L. Anderson, Effects of adsorbed polyelectrolytes on convective flow and diffusion in porous membranes, J. Membr. Sci. 28 (1986) 269–286.
[70] L.E. Bromberg, Transport of monocharged ions through environment-sensitive composite membranes based on polyelectrolyte complexes, J. Membr. Sci. 62 (1991) 117–130.
[71] H. Feil, Y.H. Bae, J. Feijen, S.W. Kim, Molecular separation by thermosensitive hydrogel membranes, J. Membr. Sci. 64 (1991) 283–294.
[72] K.P. Antonsen, J.L. Bohnert, Y. Nabeshima, M.S. Sheu, X.S. Su, A.S. Hoffman, Controlled release of proteins from 2-hydroxyethyl methacrylate copolymer gels, Biomater. Artif. Cells Immobilization Biotechnol. 21 (1993) 1–22.
[73] E. Kokufuta, Novel applications for stimulus-sensitive polymer gels in the preparation of functional immobilized biocatalysts, Adv. Polym. Sci. 110 (1993) 157–177.
[74] S.W. Kim, Y.H. Bae, T. Okano, Hydrogels: Swelling, drug loading, and release, Pharm. Res. 9 (1992) 283–290.
[75] S.Z. Song, S.H. Kim, J.R. Cardinal, S.W. Kim, Progestin permeation through polymer membranes. V. Progesterone release from monolithic hydrogel devices, J. Pharm. Sci. 70 (1981) 216–219.
[76] S.H. Gehrke, L. Uhden, M.E. Schiller, Enhanced loading and activity retention of proteins in hydrogel delivery systems, in: Proceedings of the International Symposium on the Controlled Release of Bioactive Materials, Controlled Release Society, 22, 1995, pp. 145–146.
[77] L. Bromberg, Crosslinked poly(ethylene glycol) networks as reservoirs for protein delivery, J. Appl. Polym. Sci. 59 (1996) 459–466.
[78] J.T. Chin, S.L. Wheeler, A.M. Klibanov, On protein solubility in organic solvents, Biotechnol. Bioeng. 44 (1994) 140–145.
[79] J. Matsuura, M.E. Powers, M.C. Manning, E. Shefter, Structure and stability of insulin dissolved in 1-octanol, J. Am. Chem. Soc. 115 (1993) 1261–1264.
[80] L.E. Bromberg, A.M. Klibanov, Detergent-enabled transport of proteins and nucleic acids through hydrophobic solvents, Proc. Natl. Acad. Sci. USA 91 (1994) 143–147.
[81] L.E. Bromberg, A.M. Klibanov, Transport of proteins dissolved in organic solvents through biomimetic membranes, Proc. Natl. Acad. Sci. USA 92 (1995) 1262–1266.
[82] S.H. Gehrke, J. Robeson, J. Johnson, N. Vaid, Protein isolation by solution-controlled gel sorption, Biotech. Prog. 7 (1991) 355–358.
[83] A.H. Muhr, J.M.V. Blanshard, Diffusion in gels, Polymer 23 (1982) 1012–1026.
[84] P.J. Flory, Principles of Polymer Chemistry, 15th ed., Cornell University Press, Ithaca, 1992.
[85] P.G. de Gennes, Scaling Concepts in Polymer Physics, Cornell University Press, Ithaca, 1979.
[86] T. Tanaka, Phase transitions in gels and a single polymer, Polymer 20 (1979) 1404–1412.
[87] T. Tanaka, D. Fillmore, S.-T. Sun, I. Nishio, G. Swislow, A. Shah, Phase transitions in ionic gels, Phys. Rev. Lett. 45 (1980) 1636–1639.
[88] I. Ohmine, T. Tanaka, Salt effects on the phase transition of ionic gels, J. Chem. Phys. 77 (1982) 5725–5729.
[89] D. Nicoli, C. Young, T. Tanaka, A. Pollak, G. Whitesides, Chemical modification of acrylamide gels: Verification of the role of ionization in phase transitions, Macromolecules 16 (1983) 887–890.
[90] L. Bromberg, A.Yu. Grosberg, E.S. Matsuo, Y. Suzuki, T. Tanaka, Dependency of swelling on the length of subchain in poly(*N*,*N*-dimethylacrylamide) gels, J. Chem. Phys. 106 (1997) 2906–2911.
[91] Y. Li, T. Tanaka, Study of the universality class of the gel network system, J. Chem. Phys. 90 (1989) 5161–5166.
[92] I.C. Sanchez, R.H. Lacombe, Statistical thermodynamics of polymer solutions, Macromolecules 11 (1978) 1145–1156.
[93] I.C. Sanchez, R.H. Lacombe, An elementary molecular theory of classical fluids. Pure Fluids, J. Phys. Chem. 80 (1976) 2352–2362.
[94] R.H. Lacombe, I.C. Sanchez, Statistical thermodynamics of fluid mixtures, J. Phys. Chem. 80 (1976) 2568–2580.
[95] M. Marchetti, S. Prager, E.L. Cussler, Thermodynamic predictions of volume changes in temperature-sensitive gels. 1. Theory, Macromolecules 23 (1990) 1760–1765.
[96] M. Marchetti, S. Prager, E.L. Cussler, Thermodynamic predictions of volume changes in temperature-sensitive gels. 2. Experiments, Macromolecules 23 (1990) 3445–3450.
[97] K.K. Lee, E.L. Cussler, M. Marchetti, M.A. McHugh, Pressure-dependent phase transitions in hydrogels, Chem. Eng. Sci. 45 (1990) 766–767.
[98] H.H. Hooper, H.W. Blanch, J.M. Prausnitz, Configurational properties of partially ionized polyelectrolytes from Monte Carlo simulation, Macromolecules 23 (1990) 4820–4829.
[99] S. Beltran, H.H. Hooper, H.W. Blanch, J.M. Prausnitz, Swelling equilibria for ionized temperature-sensitive gels in water and in aqueous solution, J. Chem. Phys. 92 (1990) 2061–2066.
[100] S. Beltran, J.P. Baker, H.H. Hooper, H.W. Blanch, J.M. Prausnitz, Swelling equilibria for weakly-ionizable, tem-

perature-sensitive hydrogels, Macromolecules 24 (1990) 549–551.
[101] H. Yasuda, A. Peterlin, C.K. Colton, K.A. Smith, E.W. Merrill, Permeability of solutes through hydrated polymer membranes, Die Makromolekulare Chem. 126 (1969) 177–186.
[102] S. Wisniewski, S.W. Kim, Permeation of water-soluble solutes through poly(2-hydroxyethyl methacrylate) and poly(2-hydroxyethyl methacrylate) crosslinked with ethylene glycol dimethacrylate, J. Membr. Sci. 6 (1980) 299–308.
[103] L.E. Bromberg, A.R. Rudman, N.A. Vengerova, B.S. Eltsefon, Relation between transport properties and state of water in regenerated cellulose membranes, in: B. Sedlacek, J. Kahovec (Eds.), Synthetic Polymeric Membranes, de Gruyter, Berlin, 1987, pp. 397–402.
[104] L.E. Bromberg, B.S. Eltsefon, Mechanism of water transport through hydrate cellulose membranes, Colloid J. USSR 51 (1989) 477–480.
[105] M. Palasis, S.H. Gehrke, Permeability of responsive poly(*N*-isopropyl acrylamide) gels to solutes, J. Control. Release 18 (1992) 1–12.
[106] K.P. Rao, R. Jeyanthi, Controlled peptide delivery: Current status and future prospects, Indian J. Chem., Sect. B 30B (1991) 107–117.
[107] L.C. Dong, Q. Yan, A.S. Hoffman, Controlled release of amylase from a thermal and pH-sensitive, macroporous hydrogel, J. Control. Release 19 (1992) 171–177.
[108] J.-C. Janson, J.-Å. Jönsson, Introduction to chromatography, in: J.-C. Janson, L. Rydén (Eds.), Protein Purification. Principles, High Resolution Methods, and Applications, VCH, New York, 1989, pp. 35–62.
[109] D.H. Wertz, H.A. Sheraga, Influence of water on protein structure. An analysis of the preferences of amino acid residues for the inside or outside and for specific conformations in a protein molecule, Macromolecules 11 (1978) 9–15.
[110] J.L. Cornette, K.B. Cease, H. Margalit, J.L. Spouge, J.A. Berzofsky, C. DeLisi, Hydrophobicity scales and computational techniques for detecting amphipathic structures in proteins, J. Mol. Biol. 195 (1987) 659–685.
[111] S.L. Wu, A. Figueroa, B.L. Karger, Protein conformational effects in hydrophobic interaction chromatography. Retention characterization and the role of mobile phase additives and stationary phase hydrophobicity, J. Chromatogr. 371 (1986) 3–27.
[112] A. Domb, G.W.R. Davidson, L.M. Sanders, Diffusion of peptides through hydrogel membranes, J. Control. Release 14 (1990) 133–144.
[113] T.G. Park, A.S. Hoffman, Immobilization of Arthrobacter simplex in thermally reversible hydrogel: Effect of gel hydrophobicity on steroid conversion, Biotech. Prog. 7 (1991) 383–390.
[114] G.M. Zentner, J.R. Cardinal, J. Feijen, S.-Z. Song, Progestin permeation through polymer membranes. IV: Mechanism of steroid permeation and functional group contributions to diffusion through hydrogel films, J. Pharm. Sci. 68 (1979) 970–975.
[115] L.E. Bromberg, Transport of ions through heterogeneous membranes: Diffusion in the volume and on the surface of pores, Russ. J. Phys. Chem. 64 (1990) 1022–1028.
[116] V. Mitlin, L. Bromberg, Analysis of diffusion through composite membranes–I. Mathematical development, Chem. Eng. Sci. 47 (1992) 695–703.
[117] N.I. Nikolaev, Diffusion in Membranes, Khimiya, Moscow, 1980, pp. 192–196.
[118] S. Hirotsu, Coexistence of phases and the nature of first-order phase transition in poly(*N*-isopropylacrylamide) gels, Adv. Polym. Sci. 110 (1993) 1–26.
[119] M. Irie, Stimuli-responsive poly(*N*-isopropylacrylamide). Photo- and chemical-induced phase transitions, Adv. Polym. Sci. 110 (1993) 49–65.
[120] J. Eliassaf, Aqueous solutions of poly(*N*-isopropylacrylamide), J. Appl. Polym. Sci. 22 (1978) 873–874.
[121] H.G. Schild, D.A. Tirrell, Microcalorimetric detection of lower critical solution temperatures in aqueous polymer solutions, J. Phys. Chem. 94 (1990) 4352–4356.
[122] I.Yu. Galaev, B. Mattiasson, Thermoreactive water soluble polymers, nonionic surfactants, and hydrogels as reagents in biotechnology, Enzyme Microb. Technol. 15 (1993) 345–366.
[123] S. Hirotsu, Y. Hirokawa, T. Tanaka, Volume-phase transitions of ionized *N*-isopropylacrylamide gels, J. Chem. Phys. 87 (1987) 1392–1395.
[124] M. Ilavsky, J. Hrouz, I. Havlicek, Phase transition in swollen gels: 7. Effect of charge concentration on the temperature collapse of poly(*N*,*N*-diethylacrylamide) networks in water, Polymer 26 (1985) 1514–1518.
[125] R. Freitag, T. Baltes, M. Eggert, A comparison of thermoreactive water-soluble poly-*N*,*N*-diethylacrylamide prepared by anionic and by group transfer polymerization, J. Polym. Sci., Part A: Polym. Chem. 32 (1994) 3019–3030.
[126] L. Bromberg, G. Levin, Ion-selective gel that is sensitive to temperature, pH, and redox reactions, Macromol. Rapid Commun. 17 (1996) 169–172.
[127] A. Mamada, T. Tanaka, D. Kungwatchakun, M. Irie, Photoinduced phase transition of gels, Macromolecules 23 (1990) 1517–1519.
[128] A. Suzuki, T. Tanaka, Phase transition in polymer gels induced by visible light, Nature 346 (1991) 345–347.
[129] H. Ringsdorf, J. Venzmer, F.M. Winnik, Fluorescence studies of hydrophobically modified poly(*N*-isopropylacrylamides), Macromolecules 24 (1991) 1678–1686.
[130] Y. Hirose, T. Amiya, Y. Hirokawa, T. Tanaka, Phase transition of submicron gel beads, Macromolecules 20 (1987) 1342–1344.
[131] A. Suzuki, Phase transition in gels of sub-millimeter size induced by interaction with stimuli, Adv. Polym. Sci. 110 (1993) 199–240.
[132] A. Serres, M. Baudyš, S.W. Kim, Temperature and pH-sensitive polymers for human calcitonin delivery, Pharm. Res. 13 (1996) 196–201.
[133] M. Baudyš, A. Serres, C. Ramkissoon, S.W. Kim, Tempera-

ture and pH-sensitive polymers for polypeptide drug delivery, in: N. Ogata, S.W. Kim, J. Feijen, T. Okano (Eds.), Advanced Biomaterials in Biomedical Engineering and Drug Delivery Systems, Springer, Tokyo, 1996, pp. 112–115.

[134] C.S. Brazel, N.A. Peppas, Temperature- and pH-sensitive hydrogels for controlled release of heparin and streptokinase, in: A.G. Mikos, R.M. Murphy, H. Bernstein, N.A. Peppas (Eds.), Biomaterials for Drug and Cell Delivery, Materials Research Society, Pittsburgh, 1994, pp. 211–216.

[135] C.S. Brazel, N.A. Peppas, Pulsatile and local delivery of thrombolytic and antithrombotic agents using poly(*N*-isopropylacrylamide-*co*-methacrylic acid) hydrogels, J. Control. Release 39 (1996) 57–64.

[136] T. Aoki, M. Kawashima, H. Katono, K. Sanui, N. Ogata, T. Okano, Y. Sakurai, Temperature-responsive interpenetrating polymer networks constructed with poly(acrylic acid) and poly(*N*,*N*-dimethylacrylamide), Macromolecules 27 (1994) 947–952.

[137] L.H. Sperling, Interpenetrating Polymer Networks and Related Materials, Plenum Press, New York, 1981.

[138] F. Ilmain, T. Tanaka, E. Kokufuta, Volume transition in a gel driven by hydrogen bonding, Nature 349 (1991) 400–401.

[139] A.B. Scranton, J. Klier, C.L. Aronson, Complexation of polymeric acids with polymeric bases, in: R.S. Harland, R.K. Prud'homme (Eds.), Polyelectrolyte Gels, ACS Symposium Series, Vol. 480, American Chemical Society, Washington, 1992, Chapter 11.

[140] B.G. Kabra, M.K. Akhtar, S.H. Gehrke, Volume change kinetics of temperature-sensitive poly(vinyl methyl ether) gel, Polymer 33 (1992) 990–995.

[141] D.C. Harsh, S.H. Gehrke, Controlling the swelling characteristics of temperature-sensitive cellulose ether hydrogels, J. Control. Release 17 (1991) 175–186.

[142] S.H. Gehrke, Synthesis, equilibrium swelling, kinetics, permeability and applications of environmentally responsive gels, Adv. Polym. Sci. 110 (1992) 81–144.

[143] M.E. Schiller, X. Yu, E.C. Lupton, Jr., E.J. Roos, H.A. Holman, E.S. Ron, Engineered Response™ hydrogels for medical applications, in: Proceedings of the International Symposium on the Controlled Release of Bioactive Materials, Controlled Release Society, 1995, 22, pp. 30–31.

[144] J.R. Crison, P.R. Siersma, M.D. Taylor, M.E. Schiller, E.S. Ron, G.L. Amidon, Release of ibuprofen, acetaminophen and phenylpropanolamine from pH Engineered Response™ hydrogels, in: Proceedings of the International Symposium on the Controlled Release of Bioactive Materials, Controlled Release Society, 22, 1995, pp. 354–355.

[145] Y.W. Chien, Novel Drug Delivery Systems, 2nd ed., Marcel Dekker, New York, 1992, p. 140.

[146] E.W. Merrill, R.W. Pekala, N.A. Mahmud, Hydrogel for blood contact, in: N.A. Peppas (Ed.), Hydrogels in Medicine and Pharmacy, Vol. III, CRC Press, Boca Raton, 1987, p. 9.

[147] A. Silberberg, Gelled aqueous systems, in: J.E. Glass (Ed.), Polymers in Aqueous Media. Performance Through Association, Advances in Chemistry Series, Vol. 223, American Chemical Society, Washington, 1989, pp. 1–14.

[148] J.-M. Guenet, Thermoreversible Gelation of Polymers and Biopolymers, Academic Press, London, 1992.

[149] A. Silberberg, P.F. Mijnlief, Study of reversible gelation of partially neutralized poly(methacrylic acid) by viscoelastic measurements, J. Polym. Sci., Part A-2 8 (1970) 1089–1110.

[150] J. Eliassaf, A. Silberberg, The gelation of aqueous solutions of polymethacrylic acid, Polymer 3 (1962) 555–564.

[151] A. Silberberg, M. Hennenberg, Relaxation of stored mechanical stress along chemical reaction pathways, Nature 312 (1984) 746–748.

[152] P.G. Righetti, R.S. Snyder, Thermally reversible gels in electrophoresis. I: Matrix characterization, Appl. Theor. Electrophoresis 1 (1988) 53–58.

[153] T. Inoue, K. Osaki, Rheological properties of poly(vinyl alcohol)/sodium borate aqueous solutions, Rheol. Acta 32 (1993) 550–555.

[154] L. Bromberg, E.C. Lupton, M.E. Schiller, M.J. Timm, G.W. McKinney, M. Orkisz, B. Hand, Responsive polymer networks and method of their use, Int. Pat. Appl. WO 97/00275, 1997.

[155] B. Vernon, S.W. Kim, Y.H. Bae, In vitro insulin release of rat islets entrapped in thermally reversible polymer gel, in: Proceedings of the International Symposium on the Controlled Release of Bioactive Materials, Controlled Release Society, 23, 1996, pp. 216–217.

[156] S. Miyazaki, S. Takeuchi, C. Yokouchi, M. Takada, Pluronic F-127 gels as a vehicle for topical administration of anticancer agents, Chem. Pharm. Bull. 32 (1984) 4205–4208.

[157] K. Morikawa, F. Okada, M. Hosokawa, H. Kobayashi, Enhancement of therapeutic effects of recombinant interleukin-2 on a transplantable rat fibrosarcoma by the use of a sustained release vehicle, Pluronic gel, Cancer Res. 47 (1987) 37–41.

[158] A.S. Hoffman, G. Chen, Block and graft copolymers and methods relating thereto, Int. Pat. Appl. WO 95/24430, 1995.

[159] A.S. Hoffman, G.H. Chen, S.Y. Kaang, Z.L. Ding, K. Randeri, B. Kabra, Novel bioadhesive, pH- and temperature-sensitive graft copolymers for prolonged mucosal drug delivery, in: N. Ogata, S.W. Kim, J. Feijen, T. Okano (Eds.), Advanced Biomaterials in Biomedical Engineering and Drug Delivery Systems, Springer, Tokyo, 1996, pp. 62–66.

[160] A.S. Hoffman, G. Chen, S. Kaang, D.T. Priebe, New bioadhesive polymer compositions for prolonged drug release in the eye, in: Proceedings of the International Symposium on the Controlled Release of Bioactive Materials, Controlled Release Society, 22, 1995, pp. 159–160.

[161] G. Chen, A.S. Hoffman, E.S. Ron, Novel hydrogels of a temperature-sensitive Pluronic® grafted to a bioadhesive polyacrylic acid backbone for vaginal drug delivery, in: Proceedings of the International Symposium on the Controlled Release of Bioactive Materials, Controlled Release Society, 22, 1995, pp. 167–168.

[162] A.S. Hoffman, G. Chen, X. Wu, Z. Ding, B. Kabra, K. Randeri, M. Schiller, E. Ron, N.A. Peppas, C. Brazel, Graft copolymers of PEO–PPO–PEO triblock polyethers on bioadhesive polymer backbones: Synthesis and properties, Polym. Prepr. 38 (1997) 524–525.

[163] BASF Performance Chemicals, FDA and EPA status, BASF Corporation, North Mount Olive, New Jersey, 1993.

[164] P. Alexandridis, T.A. Hatton, Poly(ethylene oxide)–poly-(propylene oxide)–poly(ethylene oxide) block copolymer surfactants in aqueous solutions and at interfaces: Thermodynamics, structure, dynamics, and modeling, Colloid Surfaces A 96 (1995) 1–46.

[165] P. Wang, T.P. Johnston, Kinetics of sol-to-gel transition for Poloxamer polyols, J. Appl. Polym. Sci. 43 (1991) 283–292.

[166] P. Alexandridis, Amphiphilic copolymers and their applications, Curr. Opin. Colloid Interface Sci. 1 (1996) 490–501.

[167] P.N. Hunter, P. Alexandridis, T.A. Hatton, Solubilization in amphiphilic copolymer solutions, in: S.D. Christian, J.F. Scamehorn (Eds.), Solubilization in Surfactant Aggregates, Marcel Dekker, New York, 1995, pp. 191–235.

[168] A.V. Kabanov, E.V. Batrakova, N.S. Melik-Nubarov, N.A. Fedoseev, T.Yu. Dorodnich, V.Yu. Alakhov, V.P. Chekhonin, I.R. Nazarova, V.A. Kabanov, A new class of drug carriers: Micelles of poly(oxyethylene)–poly(oxypropylene) block copolymers as microcontainers for drug targeting from blood in brain, J. Control. Release 22 (1992) 141–158.

[169] V.Yu. Alakhov, A.V. Kabanov, P.G. Sveshnikov, E.S. Severin, Composition of antineoplastic agents incorporated in micelles, PCT WO 94/08564, 1994.

[170] A.V. Kabanov, I.R. Nazarova, I.V. Astafieva, E.V. Batrakova, V.Yu. Alakhov, A.A. Yaroslavov, V.A. Kabanov, Micelle formation and solubilization of fluorescent probes in poly-(oxyethylene-*b*-oxypropylene-*b*-oxyethylene) solutions, Macromolecules 28 (1995) 2303–2314.

[171] V.Yu. Alakhov, E.Yu. Moskaleva, E.V. Batrakova, A.V. Kabanov, Hypersensitization of multidrug resistant human ovarian carcinoma cells by Pluronic P85 block copolymer, Bioconjugate Chem. 7 (1996) 209–216.

[172] V.I. Slepnev, L.E. Kuznetsova, A.N. Gubin, E.V. Batrakova, V.Yu. Alakhov, A.V. Kabanov, Micelles of poly(oxyethylene)–poly(oxypropylene) block copolymer (Pluronic) as a tool for low-molecular compound delivery into a cell. Phosphorylation of intracellular proteins with micelle incorporated [$\gamma$-$^{32}$P]ATP, Biochem. Int. 26 (1992) 587–595.

[173] A.V. Kabanov, V.I. Slepnev, L.E. Kuznetsova, E.V. Batrakova, V.Yu. Alakhov, N.S. Melik-Nubarov, P.G. Sveshnikov, V.A. Kabanov, Pluronic micelles as a tool for low-molecular compound vector delivery into a cell: Effect of *Staphylococcus aureus* enterotoxin B on cell loading with micelle incorporated fluorescent dye, Biochem. Int. 26 (1992) 1035–1040.

[174] A. Mizrahi, Pluronic polyols in human lymphocyte cell line cultures, J. Clin. Microbiol. 2 (1975) 11–18.

[175] A. Venne, S.M. Li, R. Mandeville, A. Kabanov, V. Alakhov, Hypersensitizing effect of Pluronic L61 on cytotoxic activity, Cancer Res. 56 (1996) 3626–3629.

[176] R. Paradis, C. Noel, M. Page, Use of pluronic micelles to overcome multidrug resistance, Int. J. Oncol. 5–6 (1994) 1305–1308.

[177] J.-H. Lee, J. Kopecek, J.D. Andrade, Protein-resistant surfaces prepared by PEO-containing block copolymer surfactants, J. Biomed. Mater. Res. 23 (1989) 351–368.

[178] M. Amiji, K. Park, Prevention of protein adsorption and platelet adhesion on surfaces by PEO/PPO/PEO triblock copolymers, Biomaterials 13 (1992) 682–692.

[179] J. Lee, P.A. Martic, J.S. Tan, Protein adsorption on Pluronic copolymer-coated polystyrene particles, J. Colloid Interface Sci. 131 (1989) 252–266.

[180] S.M. Moghimi, Mechanisms regulating body distribution of nanospheres conditioned with pluronic and tetronic block co-polymers, Adv. Drug Deliv. Rev. 16 (1995) 183–193.

[181] G.S. Kwon, K. Kataoka, Block copolymer micelles as long-circulating drug vehicles, Adv. Drug Deliv. Rev. 16 (1995) 295–309.

[182] J.L. Haslam, T. Higuchi, A.R. Mlodozeniec, Ophthalmic drug delivery system utilizing thermosetting gels, U.S. Pat. 4,474,751, 1984.

[183] T.X. Viegas, L.E. Reeve, R.L. Henry, Method of making thermoreversible polyoxyalkylene gels, U.S. Pat. 5,593,683, 1997.

[184] A. Al-Saden, A.J. Florence, T.L. Whateley, Novel Poloxamer and Poloxamine hydrogels: Swelling and drug release, J. Pharm. Pharmacol. 32 (1980) 5–11.

[185] A.A. Al-Saden, A.T. Florence, T.L. Whateley, Cross-linked hydrophilic gels from ABA block copolymeric surfactants, Int. J. Pharm. 5 (1980) 317–327.

[186] A. Joshi, S. Ding, K.J. Himmelstein, Reversible gelation compositions and methods of use, U.S. Pat. 5,252,318, 1993.

[187] S. Kumar, B.O. Haglund, K.J. Himmelstein, In situ-forming gels for ophthalmic drug delivery, J. Ocular Pharmacol. 10 (1994) 47–56.

[188] M. Gibson, P.M. Taylor, N.I. Payne, P.L. Gould, Pharmaceutical compositions useful as drug delivery vehicles and/or as wound dressings, Eur. Pat. Appl. EP 0,386,960, 1990.

[189] A.L. Hoeg, D.L. Meadows, Reversible gelation emulsion compositions and methods of use, U.S. Pat. 5,441,732, 1995.

[190] BASF Performance Chemicals, Pluronic® and Tetronic® Surfactants, BASF Corporation, North Mount Olive, New Jersey, 1996.

[191] P. Linse, Micellization of poly(ethylene oxide)–poly-(propylene oxide) block copolymers in aqueous solution, Macromolecules 26 (1993) 4437–4449.

[192] P. Linse, M. Malmsten, Temperature-dependent micellization in aqueous block copolymer solutions, Macromolecules 25 (1992) 5434–5439.

[193] P. Alexandridis, J.F. Holzwarth, T.A. Hatton, Micellization of poly(ethylene oxide)–poly(propylene oxide)–poly-(ethylene oxide) triblock copolymers in aqueous solutions: Thermodynamics of copolymer association, Macromolecules 27 (1994) 2414–2425.

[194] K. Zhang, A. Khan, Phase behavior of poly(ethylene

oxide)–poly(propylene oxide)–poly(ethylene oxide) triblock copolymers in water, Macromolecules 28 (1995) 3807–3812.

[195] N. Almgren, W. Brown, S. Hvidt, Self-aggregation and phase behavior of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) block copolymers in aqueous solution, Colloid Polym. Sci. 273 (1995) 2–15.

[196] B. Chu, Z. Zhou, Physical chemistry of polyoxyalkylene block copolymers, Surfactant Sci. Ser. 60 (1996) 67–144.

[197] P. Alexandridis, T. Nivaggioli, T.A. Hatton, Temperature effects on structural properties of pluronic P104 and F108 PEO–PPO–PEO block copolymer solutions, Langmuir 11 (1995) 1468–1476.

[198] R.J. Holland, E.J. Parker, K. Guiney, F.R. Zeld, Fluorescence probe studies of ethylene oxide/propylene oxide block copolymers in aqueous solution, J. Phys. Chem. 99 (1995) 11981–11988.

[199] R.G. Laughlin, The Aqueous Phase Behavior of Surfactants, Academic Press, London, 1994.

[200] G. Wanka, H. Hoffmann, W. Ulbricht, Phase diagrams and aggregation behavior of poly(oxyethylene)–poly(oxypropylene)–poly(oxyethylene) triblock copolymers in aqueous solutions, Macromolecules 27 (1994) 4145–4159.

[201] P. Alexandridis, D. Zhou, A. Khan, Lyotropic liquid crystallinity in amphiphilic block copolymers: Temperature effects on phase behavior and structure of poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) copolymers of different compositions, Langmuir 12 (1996) 2690–2700.

[202] S.C. Miller, M.D. Donovan, Effect of poloxamer 407 gel on the miotic activity of pilocarpine nitrate in rabbits, Int. J. Pharm. 12 (1982) 147–152.

[203] E. Duliba, G. Lowther, The safety and efficacy of a new contact lens cleaner/rinse solution containing the surfactant pluronic 17R4: A review, Today's Ther. Trends 13 (1995) 39–46.

[204] S. Miyazaki, T. Tobiyama, M. Takada, D. Attwood, Percutaneous absorption of indomethacin from pluronic F127 gels in rats, J. Pharm. Pharmacol. 47 (1995) 455–457.

[205] K.A. Gates, H. Grad, P. Birek, P.I. Lee, A new erodible polymer insert for the controlled release of metronidazole, Pharm. Res. 11 (1994) 1605–1609.

[206] X. Xu, P.I. Lee, Programmable drug delivery from an erodible association polymer system, Pharm. Res. 10 (1993) 1144–1152.

[207] M. Guzman, F.F. Garcia, J. Molpeceres, M.R. Abeturas, Polyoxyethylene–polyoxypropylene block copolymer gels as sustained release vehicles for subcutaneous drug administration, Int. J. Pharm. 80 (1992) 119–127.

[208] T.G. Park, S. Cohen, R. Langer, Controlled protein release from polyethyleneimine-coated poly(L-lactic acid)/Pluronic blend matrices, Pharm. Res. 9 (1992) 37–39.

[209] T.G. Park, S. Cohen, R. Langer, Poly(L-lactic acid)/Pluronic blends: Characterization of phase separation behavior, degradation, and morphology and use as protein-releasing matrices, Macromolecules 25 (1992) 116–122.

[210] J.C. Gilbert, M. Whiteman, Controlled drug release from pluronic matrices, J. Pharm. Pharmacol. Suppl. 42 (1990) 128.

[211] J.C. Gilbert, J.L. Richardson, M.C. Davies, K.J. Palin, J. Hadgraft, The effect of solutes and polymers on the gelation properties of Pluronic F-127 solutions for controlled drug delivery, J. Control. Release 5 (1987) 113–118.

[212] S.G. Frank, P.C. Chenchow, F. Sadaka, Pluronic F-127 gels as potential topical drug delivery systems, Acta Pharm. Suec. 20 (1983) 30–31.

[213] H. Tomida, M. Shinohara, N. Kuwada, S. Kiryu, In vitro release characteristics of diclofenac and hydrocortisone from Pluronic F-127 gels, Acta Pharm. Suec. 24 (1987) 263–272.

[214] J. Hadgraft, J.R. Howard, Drug release from pluronic gels, J. Pharm. Pharmacol. Suppl. 34 (1982) 3.

[215] W. Brown, K. Schillen, S. Hvidt, Triblock copolymers in aqueous solution studied by static and dynamic light scattering and oscillatory shear measurements. Influence of relative block sizes, J. Phys. Chem. 96 (1992) 6038–6044.

[216] W. Brown, K. Schillen, M. Almgren, S. Hvidt, P. Bahadur, Micelle and gel formation in a poly(ethylene oxide)/poly-(propylene oxide)/poly(ethylene oxide) triblock copolymer in water solution. Dynamic and static light scattering and oscillatory shear measurements, J. Phys. Chem. 95 (1991) 1850–1858.

[217] M. Malmsten, B. Lindman, Self-assembly in aqueous block copolymer solutions, Macromolecules 25 (1992) 5440–5445.

[218] M. Malmsten, B. Lindman, Water self-diffusion in aqueous block copolymer solutions, Macromolecules 25 (1992) 5446–5450.

[219] K. Zhang, B. Lindman, L. Coppola, Melting of block copolymer self-assemblies induced by a hydrophilic surfactant, Langmuir 11 (1995) 538–542.

[220] M. Almgren, J. Van Stam, C. Lindblad, P. Li, P. Stilbs, P. Bahadur, Aggregation of poly(ethylene oxide)–poly-(propylene oxide)–poly(ethylene oxide) triblock copolymers in the presence of sodium dodecyl sulfate in aqueous solution, J. Phys. Chem. 95 (1991) 5677–5684.

[221] M. Malmsten, B. Lindman, Effects of homopolymers on the gel formation in aqueous block copolymer solutions, Macromolecules 26 (1993) 1282–1286.

[222] D. Attwood, J.H. Colett, C.J. Tait, The micellar properties of the poly(oxyethylene)–poly(oxypropylene) copolymer Pluronic F127 in water and electrolyte solution, Int. J. Pharm. 26 (1985) 25–33.

[223] J. Juhasz, V. Lenaerts, M.T. Phan Viet, H. Ong, Effect of sodium chloride on physical characteristics of Poloxamer 407 solutions, J. Colloid Interface Sci. 136 (1990) 168–174.

[224] T.P. Johnston, S.C. Miller, Toxicological evaluation of poloxamer vehicles for intramuscular use, J. Parent. Sci. Technol. 39 (1985) 83–88.

[225] T.P. Johnston, M.A. Punjabi, C.J. Froelich, Sustained delivery of interleukin-2 from a poloxamer 407 gel matrix following intraperitoneal injection in mice, Pharm. Res. 9 (1992) 425–434.

[226] E.A. Pec, Z.G. Wout, T.P. Johnston, Biological activity of urease formulated in poloxamer 407 after intraperitoneal injection in the rat, J. Pharm. Sci. 81 (1992) 626–630.
[227] K.A. Futs, T.P. Johnston, Sustained-release of urease from a poloxamer gel matrix, J. Parent Sci. Technol. 44 (1990) 58–65.
[228] P.L. Wang, T.P. Johnston, Sustained-release of interleukin-2 following intramuscular injection in the rats, Int. J. Pharm. 113 (1995) 73–81.
[229] M. Katakam, L.N. Bell, A.K. Banga, Effect of surfactants on the physical stability of recombinant human growth hormone, J. Pharm. Sci. 84 (1995) 713–716.
[230] M. Katakam, W.R. Ravis, A.K. Banga, Controlled release of human growth hormone in rats following parenteral administration of poloxamer gels, J. Control. Release. 49 (1997) 21–26.
[231] L. Brown, L. Huang, J. Dahlin, M. Moses, Ophthalmic applications of Poloxamer 407 thermal reversible gels, in: Proceedings of the American Chemical Society Conference on Formulations and Drug Delivery, Boston, 1995, p. 76.
[232] L.E. Bromberg, T.H.E. Mendum, M.J. Orkisz, E.S. Ron, E.C. Lupton, Applications of poly(oxyethylene-*b*-oxypropylene-*b*-oxyethylene)-*g*-poly(acrylic acid) polymers (Smart Hydrogel™) in drug delivery, Proc. Polym. Mater. Sci. Eng. 76 (1997) 273–275.
[233] M.J. Orkisz, L. Bromberg, R. Pike, E.C. Lupton, E.S. Ron, Rheological properties of reverse thermogelling poly(acrylic acid)-*g*-poly(oxyethylene-*b*-oxypropylene-*b*-oxyethylene) polymers (Smart Hydrogel™), Proc. Polym. Mater. Sci. Eng. 76 (1997) 276–277.
[234] E.S. Ron, E.J. Roos, A.K. Staples, L.E. Bromberg, M.E. Schiller, Interpenetrating polymer networks for sustained dermal delivery, in: Proceedings of the International Symposium on the Controlled Release of Bioactive Material, Controlled Release Society, 23, 1996, pp. 128–129.
[235] L. Bromberg, M. Orkisz, E. Roos, E.S. Ron, M. Schiller, Interpenetrating networks of Poloxamer copolymers and poly(acrylic acid) as vehicles in controlled drug delivery, in: Proceedings of the Fourth European Symposium on Controlled Release, Noordwijk aan Zee, Netherlands, 1996, pp. 126–131.
[236] E.S. Ron, E. Roos, A. Staples, M. Schiller, L. Bromberg, A new vehicle for topical and mucosal drug delivery, Pharm. Res. Suppl. 13 (1996) S299.
[237] A.M. Potts, S. Jackson, N. Washington, P. Gilchrist, E.S. Ron, M. Schiller, C.G. Wilson, In vivo determination of the oesophageal retention of Smart Hydrogel™, in: Proceedings of the International Symposium on the Controlled Release of Bioactive Materials, Controlled Release Society, 24, 1997, pp. 335–336.
[238] E.S. Ron, L. Bromberg, S. Luszak, M. Kearney, D.R. Deaver, M. Schiller, Smart Hydrogel™: A novel mucosal delivery system, in: Proceedings of the International Symposium on the Controlled Release of Bioactive Materials, Controlled Release Society, 24, 1997, pp. 407–408.
[239] E.S. Ron, L. Bromberg, S. Luszak, M. Kearney, D.R. Deaver, M. Schiller, Smart Hydrogel™: A new vehicle for mucosal drug delivery, in: Proceedings of the Eighth International Symposium on Recent Advances in Drug Delivery Systems, Salt Lake City, 1997, pp. 186–187.
[240] I.N. Topchieva, I.G. Momot, V.P. Ivanova, N.V. Efremova, Free-radical substitution reactions in ethylene oxide–propylene oxide block copolymers, Moscow Univ. Chem. Bull. 45 (1990) 95–98.
[241] Final Assessment Report of the Safety of Carbomers 934, -934P, -940, -941 and - 962, American College of Toxicology, 1982, 1, pp. 109–141.
[242] E.E. Hassan, J.M. Gallo, A simple rheological method for the in vitro assessment of mucin–polymer bioadhesive bond strength, Pharm. Res. 7 (1990) 491–495.
[243] E.S. Ron, L. Bromberg, L. Varady, K. Shackett, M. Orkisz, M. Schiller, Interpenetrating polymeric networks in protein delivery, Abstr. Third US–Japan Symposium on Drug Delivery Systems, Maui, Hawaii, 1995, No. 58.
[244] L.E. Bromberg, T.H.E. Mendum, M.J. Orkisz, E.C. Lupton, E.S. Ron, Polyoxyethylene-*b*-polyoxypropylene-*b*-polyoxyethylene-*g*-poly(acrylic acid) polymers (Smart Hydrogel™) as a carrier in controlled delivery of proteins and peptides, Polym. Prepr. 38 (1997) 602–603.
[245] Bromberg, L.E., Orkisz, M.J., Ron, E.S., Bioadhesive properties of polyoxyethylene-*b*-polyoxypropylene-*b*-polyoxyethylene-*g*-poly(acrylic acid) polymers (Smart Hydrogel™), Polym. Prepr., 38 (1997) 626-627.
[246] A.V. Kabanov, V.Yu. Alakhov, Micelles of amphiphilic block copolymers as vehicles for drug delivery, in: P. Alexandridis, B. Lindman (Eds.), Amphiphilic Block Copolymers: Self Assembly and Applications, Elsevier, Amsterdam. (in press).
[247] D.A. Edwards, K.J. Gooch, I. Zhang, G.H. McKinley, R. Langer, The nucleation of receptor-mediated endocytosis, Proc. Natl. Acad. Sci. USA 93 (1996) 1786–1791.
[248] P.S. Stayton, T. Shimoboji, C. Long, A. Chilkoti, G. Chen, J.M. Harris, A.S. Hoffman, Control of protein–ligand recognition using a stimuli-responsive polymer, Nature 378 (1995) 472–474.
[249] W.-C. Shen, J. Wan, H. Ekrami, Means to enhance penetration. Enhancement of polypeptide and protein absorption by macromolecular carriers via endocytosis and transcytosis, Adv. Drug Deliv. Rev. 8 (1992) 93–113.
[250] B. Jeong, Y.H. Bae, D.S. Lee, S.W. Kim, Biodegradable block copolymers as injectable drug-delivery systems, Nature, 388 (1997) 860–862.