

Thermosensitive Interpenetrating Polymer Networks: Synthesis, Characterization, and Macromolecular Release

Anna Gutowska, You Han Bae, Harvey Jacobs, Jan Feijen,[†] and Sung Wan Kim*

Department of Pharmaceutics and Pharmaceutical Chemistry and Center for Controlled Chemical Delivery, University of Utah, 421 Wakara Way #318, Salt Lake City, Utah 84108

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ABSTRACT: Thermosensitive semiinterpenetrating polymer networks (semi-IPNs) composed of cross-linked poly(*N*-isopropylacrylamide) (NiPAAm) and linear poly(ether(urethane-urea) (Biomer) were obtained via UV-initiated solution polymerization. The semi-IPNs exhibited negative thermosensitivity, i.e., lower swelling levels with increasing temperature. The incorporation of a relatively small content of Biomer (up to 10 wt %) strongly influenced the mechanical properties, equilibrium swelling, and deswelling kinetics of synthesized networks. The semi-IPNs exhibited greater mechanical strength compared to the cross-linked poly(NiPAAm). Equilibrium swelling levels of the semi-IPNs at low temperatures were markedly decreased due to hydrophobic contribution of Biomer and higher apparent effective cross-linking densities of these networks. The gel collapse point, related to the lower critical solution temperature of poly(NiPAAm), was not affected. The semi-IPNs showed much faster deswelling rates compared to the cross-linked poly(NiPAAm). It was hypothesized that the presence of Biomer prevented the formation of a skin-type layer which normally retards the deswelling process of cross-linked poly(NiPAAm). Loading and release of heparin, a model macromolecule, was studied as a function of temperature and Biomer content in semi-IPNs. The partition coefficients of heparin within the networks decreased with increasing temperature and Biomer content. Similarly, a linear relationship between partition coefficients and equilibrium swelling in loading solutions was found for all synthesized networks. Heparin release profiles correlated with deswelling kinetics of cross-linked poly(NiPAAm) and NiPAAm/Biomer semi-IPNs. Release profiles were in agreement with the proposed mechanism of solute release from swollen thermosensitive gels.

Introduction

Hydrogels demonstrating temperature-dependent swelling in water have been extensively studied in recent years. Negative thermosensitivity, i.e., decreasing swelling levels with increase temperatures, is observed in gels obtained by cross-linking of polymers which exhibit a lower critical solution temperature (LCST) in aqueous solutions.¹ LCST results from the influence of temperature on polymer-polymer and polymer-water interactions such as hydrogen-bonding and hydrophobic interactions. As a specific example, polymer gels composed of cross-linked poly(NiPAAm) undergo a sharp volume phase transition (gel collapse) at the temperature close to the LCST of linear polymer.² The fundamental molecular and physical properties of NiPAAm-based thermosensitive hydrogels (TSH) can be investigated and optimized for a variety of biomedical and engineering uses, such as controlled drug delivery,^{3,4} molecular separation processes⁵ concentration of macromolecular solutions,⁶ enzyme activity controlling systems,^{7,8} and tissue culture substrates.⁹

A serious limitation of pure NiPAAm gel in many of these applications is its low mechanical strength in a highly swollen state. Modification of TSH with hydrophobic components through copolymerization or formation of an IPN was shown to improve the mechanical properties.^{10,11} Copolymerization of NiPAAm with hydrophobic monomers decreased gel swelling levels as well as the gel collapse temperature. On the other hand, modification with hydrophobic components via an IPN formation affected only the equilibrium swelling levels without affecting the gel collapse temperature.¹¹

Recently, thermosensitive hydrogel IPNs (TSH-IPNs) have been synthesized using poly(NiPAAm) and poly(tetramethylene glycol)¹² or poly(ethylene oxide-dimethylsiloxane-ethylene oxide).¹³ The networks demonstrated "on-off" solute transport in response to temperature changes. TSH-IPNs composed of poly(acrylamide-co-butyl methacrylate) and poly(acrylic acid) were studied by Okano et al.¹⁴ These networks form intermolecular complexes through hydrogen bonding at low temperatures. Disruption of hydrogen bonds at higher temperatures and dissociation of polymer-polymer complexes lead to positive thermosensitivity, i.e., higher swelling levels with increasing temperature. A different TSH-IPN system based on two incompatible polymers, poly(ethylene oxide) and *N*-acryloylpyrrolidine, was studied by Bae et al.¹⁵ It was suggested that temperature-dependent polymer-polymer repulsive interactions between the two networks were responsible for the swelling force balance in the system.

Modification of hydrogels via an IPN formation is an effective method to obtain materials with more versatile properties.¹⁶ IPN formation could also be used to modify a surface of a hydrophobic polymer with hydrophilic components. For example, an interesting gradient IPN system was obtained with poly(ether urethane) and poly(acrylamide), which absorbed water in the manner of a hydrogel but had mechanical properties superior to a hydrogel.¹⁷ If only one component in the system is cross-linked, the formed network is termed a semi-IPN. Hydrogel semi-IPNs may be conveniently synthesized by polymerizing lightly cross-linked hydrophilic monomers around a hydrophobic, reinforcing linear polymer. The formation and behavior of such systems using cellulose derivatives as the reinforcing polymer and 2-hydroxyethyl methacrylate, *N*-vinylpyrrolidone, and *N,N*-dimethylacrylamide as highly swelling components were studied by Corkhill and Tighe.^{18,19}

* To whom correspondence should be addressed. Telephone: (801) 581-6654. Fax: (801) 581-7848.

[†] Department of Chemical Technology, University of Twente, Enschede, The Netherlands.

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Table 1. Feed Compositions for NiPAAm/Biomer Semi-IPNs and Cross-Linked Poly(NiPAAm)

	NiPAAm	IPN-2	IPN-3	IPN-5	IPN-7	IPN-10
Biomer wt %	0.00	2.5	3.75	5.00	7.50	10.00
NiPAAm (g)	1.00	1.00	1.00	1.00	1.00	1.00
EGDMA (μ L)	17.72	17.72	17.72	17.72	17.72	17.72
BME (g)	0.0045	0.0045	0.0045	0.0045	0.0045	0.0045
Biomer (mL)	0.00	0.50 ^a	0.40 ^b	0.55 ^b	0.80 ^b	1.11 ^b
DMAc (mL)	1.00	0.50	0.60	0.45	0.20	0.00

^a 5 wt % solution of Biomer in DMAc. ^b 10 wt % solution of Biomer in DMAc.

In this study, novel TSH semi-IPNs composed of cross-linked poly(NiPAAm) and physically entangled copoly-(ether urethane-urea) (Biomer) were synthesized via UV-initiated solution polymerization. Biomer was used as a hydrophobic polymer to enhance the mechanical strength of the otherwise weak NiPAAm gel. Biomer is a multiple block copolymer(ether urethane-urea) which has been considered for biomaterial application by numerous investigators.²⁰ The Biomer network in the semi-IPNs is not covalently cross-linked and is expected to allow for high swelling levels at temperatures below the gel collapse point. This high swelling should allow for loading of high molecular weight bioactive molecules. The subsequent release of macromolecules from the swollen gel at the temperatures above the gel collapse point is expected to be controlled by the squeezing action of the collapsing polymer network.

This paper will describe the synthesis, swelling behavior, and release properties of the NiPAAm/Biomer semi-IPNs, especially as they relate to the effect of the hydrophobic component, Biomer, on the equilibrium swelling and deswelling kinetics of the networks. Partitioning and release of a model macromolecule, heparin, was also considered as a function of temperature and network composition. Mechanisms of solute release from swollen TSH are discussed.

Materials and Methods

Materials. N-Isopropylacrylamide (NiPAAm), obtained from Eastman Kodak Co., was recrystallized from hexane. Ethylene glycol dimethylacrylate (EGDMA) was obtained from Poly-science, Inc., and purified by distillation at 60 °C (2 mmHg). Benzoin methyl ether (BME), the UV initiator, was obtained from Eastman Kodak Co. and was used as received. *N,N*-Dimethylacetamide (DMAc), glass-distilled spectral grade, was purchased from EM Science. Heparin (weight-average molecular weight, $M_w = 6000$) was obtained from Hepar, Inc., and used as received. The size distribution of heparin molecules was estimated using dynamic LLs, and a polydispersity index of $0.24 \pm 10\%$, reflecting the polydispersity of diffusion coefficients, was determined.

Solution-grade Biomer [copoly(ether urethane-urea)] was purchased from Ethicon, Inc., as a 25% solution in *N,N*-dimethylacetamide. Biomer was purified by precipitation in methanol, followed by extensive vacuum drying. Fresh Biomer solutions were prepared as 5 or 10 wt % of polymer in DMAc prior to synthesis. The weight-average molecular weight and polydispersity of Biomer were determined by Park et al.²¹ using static and dynamic laser light scattering. Due to the broad size and molecular weight distribution ($V \sim 100\%$), bimodal data analysis was used to estimate the size and fractional intensity of scattered light for "small" and "large" particles in the solution. The weight-average molecular weight of large particles was estimated to be 97 000 and that of small particles 48 000.

Synthesis of NiPAAm/Biomer Semi-IPNs. NiPAAm/Biomer semi-IPNs, containing 2.5–10 wt % of Biomer, were synthesized by UV polymerization in 50 wt % DMAc, with BME used as the initiator and EGDMA as a cross-linker for the NiPAAm network. The feed compositions of semi-IPNs are shown in Table 1. The reaction mixtures were injected between

glass plates separated by a 1.5-mm-thick rubber gasket and irradiated at room temperature (23 °C) with 365-nm UV light for 30 min. After irradiation, the gel membranes were kept in glass molds for 24 h to complete polymerization. Obtained gel membranes were purified by Soxhlet extraction with ethanol for 24 h and then soaked in water at 4 °C for 2 days. Disk-shaped samples (10 mm in diameter and 2–3 mm thick) for swelling studies were cut from membranes equilibrated in water at room temperature.

Scanning Electron Microscopy. The structural morphology of synthesized networks in the swollen state was evaluated using scanning electron microscopy (SEM). Polymer disks were swollen to equilibrium in isotonic phosphate buffered saline at 5 °C and subsequently freeze-dried at –20 °C. Samples were coated with gold and palladium (Technics Sputter Coater, Hummer III, Alexandria, VA). Cross-sectional surfaces were examined with a scanning electron microscope, JEOL JSM-35, JEOL USA, Inc., Peabody, MA.

Compression Modulus in the Swollen State. Unidirectional compression moduli of the synthesized networks were determined using a modified bench comparator (B.C. Ames Co.), as described by Cluff et al.²² Small squares (area = 1.8–2.25 cm² and thickness = 2.0–3.0 mm) were cut from membranes equilibrated in water at 23 °C. Teflon sheets were placed between the plates and samples to minimize friction and nonuniform deformation (barreling). After each measurement, the samples were placed in water for 10 min to recover their undeformed dimensions. All deformations were below 15% of the initial thickness. The compression modulus of each network was determined from the slope of the stress-strain relationship.

Cross-Linking Density. The apparent effective cross-linking densities of the synthesized networks, ν_e^* , were calculated from the stress-strain relationship using the equation:

$$\tau = RT(\phi_{p,0}/\phi_p)^{2/3}\phi_p\nu_e^*(\alpha - 1/\alpha^2)$$

where τ is the applied stress in dyn/cm², α is the linear deformation factor, and $\phi_{p,0}$ and ϕ_p are the polymer volume fractions in the relaxed state (gel after polymerization) and in the swollen state, respectively. The cross-linking efficiency of cross-linked poly-(NiPAAm) was calculated as a ratio of the experimentally determined apparent effective cross-linking density to the theoretical cross-linking density of the poly(NiPAAm) network cross-linked with 1 mol % of EGDMA.

Swelling Measurements. Equilibrium swelling properties of the synthesized networks were studied at a 5–40 °C temperature range in isotonic phosphate buffered saline (PBS), pH = 7.4. Disks were equilibrated for 3 days at a particular temperature. The equilibrium swelling ratio, S_{eq} , was calculated from the following equation:

$$S_{eq} = (W_s - W_d)/W_d$$

where W_s denotes the weight of a swollen polymer and W_d denotes the weight of a dry polymer.

For deswelling kinetic studies, gels were equilibrated in isotonic PBS at 23 °C (room temperature) to constant weight and immersed directly in PBS pre-equilibrated at 37 °C. The disks were removed from the 37 °C buffer at predetermined times, the weight was recorded, and the fractional approach to equilibrium, S_t/S_{eq} , was plotted as a function of time. S_t denotes the swelling ratio of a polymer at time t , and S_{eq} denotes the equilibrium swelling ratio at 37 °C.

Determination of Glass Transition Temperatures (T_g). T_g s of the semi-IPNs in dry state were determined by differential scanning calorimetry (DSC) using a DCS-7 Perkin-Elmer calorimeter. DSC experiments were performed in two temperature ranges, –90 to 0 and 10–150 °C, with a scan rate 10 °C/min and nitrogen used as a sweep gas. T_g s of cross-linked poly(NiPAAm) and solvent-cast Biomer films were also determined.

Heparin Loading. Heparin was loaded into NiPAAm/Biomer semi-IPNs using a solution sorption method. Three disks of each semi-IPN composition were equilibrated at a 5% heparin/isotonic PBS loading solution at 4, 15, and 23 °C (room temperature) for 1 day. Control experiments with loading times

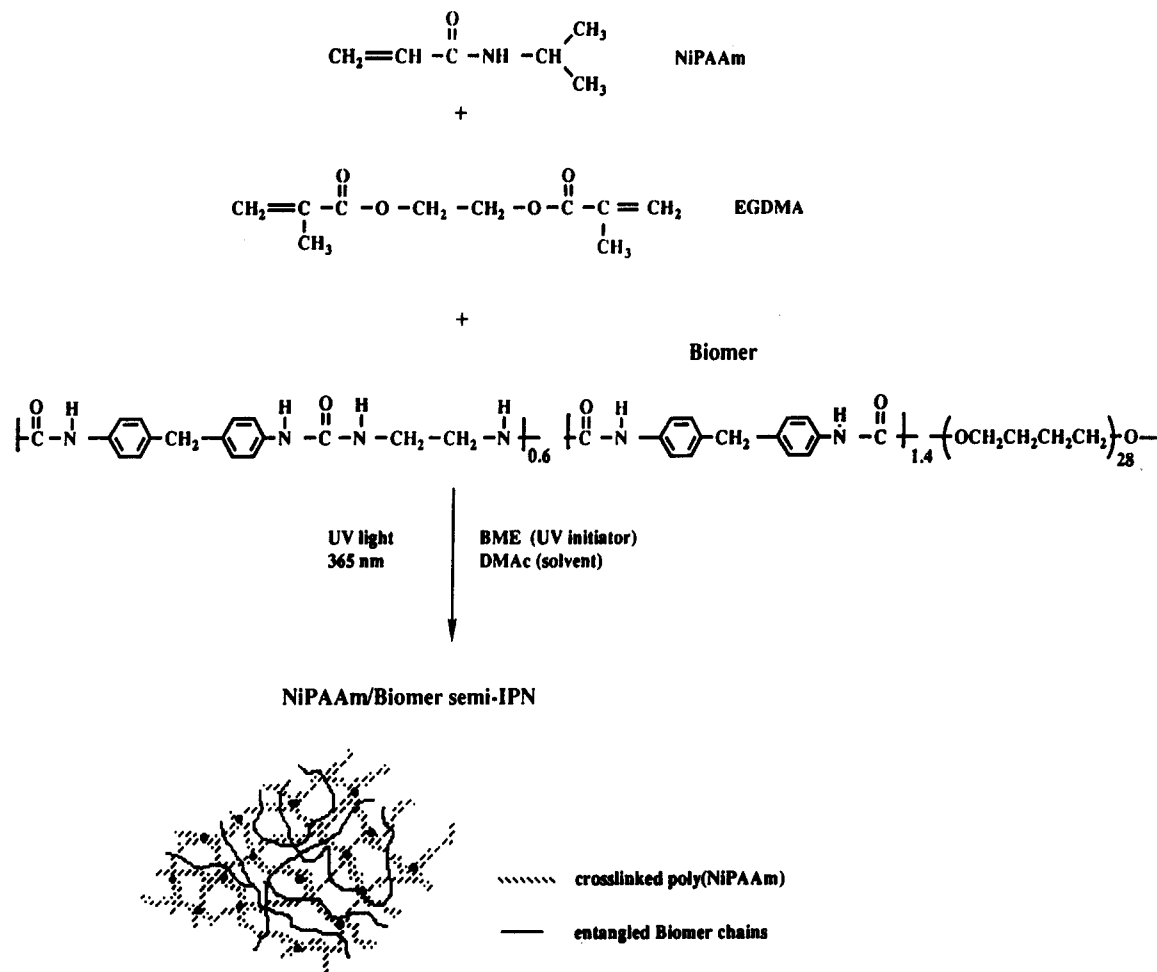


Figure 1. Schematic diagram showing components used for the synthesis, and the resulting structure of NiPAAm/Biomer semi-IPNs.

up to 14 days were also performed, and no changes in heparin loading levels were observed between 1 and 14 days. After heparin loading, the disks were briefly rinsed with PBS at the same temperature as the loading solution, the surface was blotted with a damp filter paper, and the weight of the swollen disks was measured. The concentration of heparin in the disk was determined after extensive extraction in a 4 °C buffer solution. The heparin concentration in the extracts was measured using Azure II colorimetric assay.²³

Partition coefficients, K_d , defined as the ratio of the solute concentration in the gel disk to that in the outer solution were calculated as:

$$K_d = (W_{\text{drug}}/V_{\text{disk}}) \times 100\% \times 1/C_0$$

where W_{drug} denotes the weight of heparin in the disk in grams, V_{disk} denotes the swollen volume of the disk in the loading solution, in milliliters, and C_0 denotes the heparin concentration in the loading solution % w/v. The volume of the swollen disk was estimated from weight measurements, assuming that the density of the swollen polymer is close to 1.00 g/cm³.²⁴

Heparin Release. Heparin release from synthesized networks was conducted at 37 °C in PBS at pH = 7.4. No drying procedure was applied to the loaded disks. The swollen disk was taken out of the loading solution, briefly rinsed with PBS at the temperature of the loading solution, and immersed directly in the release medium. To maintain sink conditions, disks were transferred into 10 mL of fresh PBS, pre-equilibrated at 37 °C, at pre-determined times. The amount of released heparin was measured by the Azure II colorimetric assay, described previously.²³

Results and Discussion

Synthesis and Structure. TSH semi-IPNs synthesized in this study are composed of cross-linked poly-(NiPAAm) and physically entangled hydrophobic polymer,

Biomer, that enhances the mechanical strength of the hydrogel. Biomer is a relatively biocompatible multiple block copoly(ether urethane-urea). There are two chemically distinct forms of Biomer: solution grade and extrusion grade. Figure 1 contains the chemical structure of solution-grade Biomer as determined by Lelah et al.²⁰ Solution-grade Biomer consists of soft segments of poly-(tetramethylene oxide) and hard segments identified as 4,4'-diphenylmethanediisocyanate, chain extended with diamines. Multiple block copolymers, such as Biomer, phase separate to form a two-phase morphology. Urethane-urea segments (or hard segments) form hydrogen-bonded domains, in the range of 30–50 Å, within the polyether (soft segment) matrix.²⁵

NiPAAm/Biomer semi-IPNs containing from 2.5 to 10 wt % of Biomer were synthesized via UV-initiated solution polymerization. Figure 1 shows the reaction scheme of the synthesis, and the feed compositions are summarized in Table 1. The networks formed in the synthesis were classified as semi-IPN since only one component, i.e., the poly(NiPAAm) network, was covalently cross-linked. The semi-IPN structure allowed for mixing of the two incompatible polymers, and thermosensitive hydrogels with an enhanced mechanical strength were obtained.

The ability of Biomer to reinforce the network depends on the compatibility on the molecular level. The compatibility of the components of semi-IPNs on the molecular level may be evaluated at three stages:¹⁹ (1) the initial solution of linear polymer in a monomer solvent mixture, (2) semi-IPNs in a dry state, and (3) semi-IPNs in a fully hydrated state. In the case of NiPAAm and Biomer, both components were soluble in DMAc at the concentrations

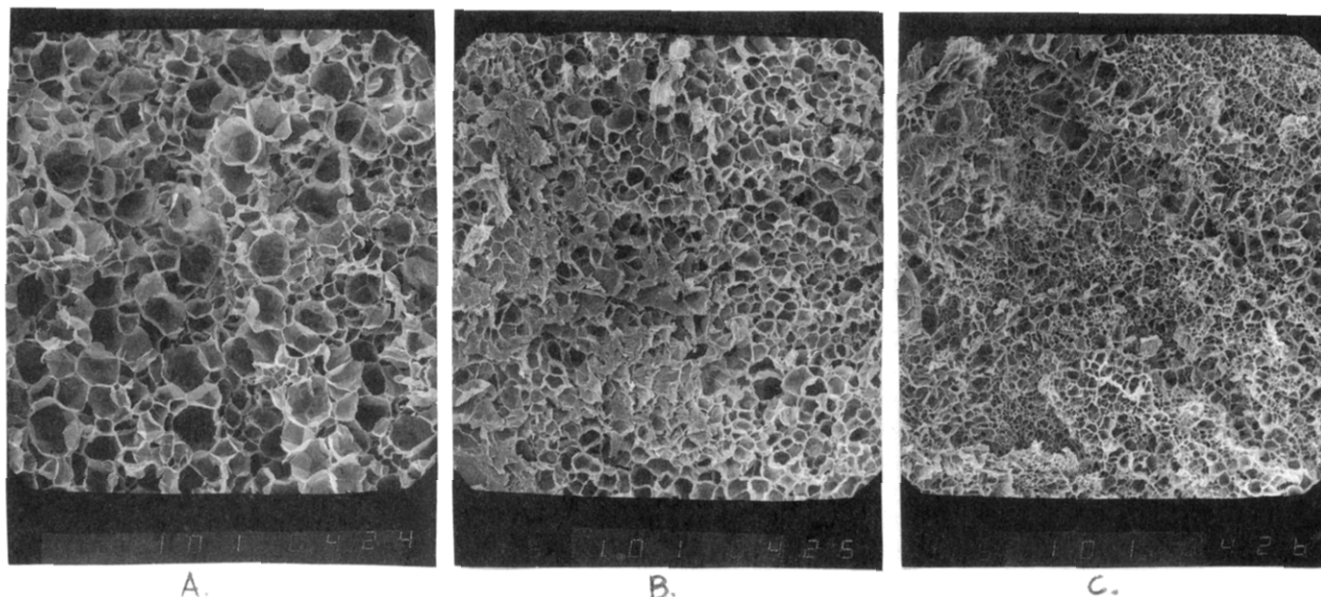


Figure 2. Scanning electron micrographs of networks swollen in PBS at 5 °C and freeze dried at -20 °C (magnification 100×): (A) cross-linked poly(NiPAAm); (B) IPN-5; (C) IPN-10.

used for the synthesis and no phase separation was observed at this stage. However, after synthesis, only the cross-linked poly(NiPAAm) was completely transparent while swollen in DMAc. All semi-IPNs swollen in DMAc showed a slight turbidity that increased with Biomer content. The same pattern was observed for all networks in the dry state. These observations suggested that some phase separation on the molecular level occurred in the dry state, as well as in the swollen state in DMAc. In the hydrated state, cross-linked poly(NiPAAm) was transparent, IPN-2 and -3 became opaque, and IPN-5, -7, and -10 turned completely white, indicating an extensive phase separation in the hydrated state, increasing with Biomer content.

The structural morphology of synthesized networks in a swollen state at low temperatures was studied using scanning electron microscopy. A cross-sectional surface area of gel disks swollen at 5 °C and freeze dried at -20 °C was examined. The structures of the poly(NiPAAm) network, IPN-5, and IPN-10 are presented in Figure 2. The porous structure of the gels is related to their high swelling levels at 5 °C. The pore size decreased with increasing Biomer content due to the networks' decreasing swelling levels.

Mechanical Properties. The mechanical properties of the networks in the swollen state were evaluated by compression modulus studies. Unidirectional compression moduli of networks equilibrated in water at 23 °C was determined using a bench comparator. Stress-strain relationships for all the networks are presented in Figure 3. Compression moduli determined from the slopes of the stress-strain curves are summarized in Table 2, together with apparent effective cross-linking densities. A relative low effective cross-linking density, and cross-linking efficiency (9%), was obtained for the cross-linked poly(NiPAAm) synthesized by UV polymerization. Higher apparent effective cross-linking densities were obtained for semi-IPNs. This is related to the fact that Biomer chains, although not covalently cross-linked, are capable of strong noncovalent interactions, such as hydrogen bonding, that may aid in the formation of physical cross-links. Hydrogen bonds can form between Biomer chains, as well as between Biomer and the poly(NiPAAm) network. Semi-IPNs with a Biomer content of 3.75 wt % and above

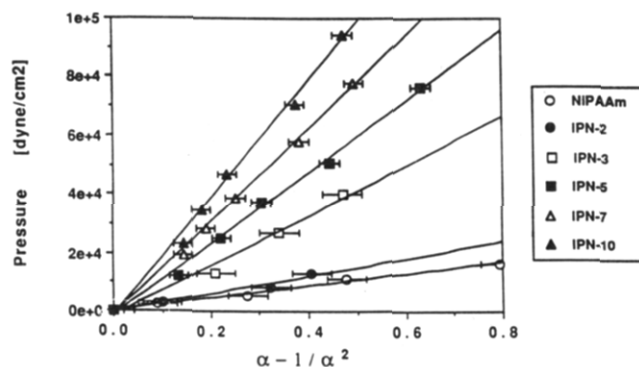


Figure 3. Stress-strain relationship of cross-linked poly(NiPAAm) and NiPAAm/Biomer semi-IPNs. Error bars represent the standard deviation for $n = 3$.

Table 2. Compression Moduli and Apparent Effective Cross-Linking Densities of Cross-Linked Poly(NiPAAm) and NiPAAm/Biomer Semi-IPNs

polymer network	biomer content, wt %	compression modulus, dyn/cm ²	apparent effective cross-linking density, mol/cm ³
NiPAAm ^a	0.00	2.1×10^4	7.97×10^{-6}
IPN-2	2.50	3.0×10^4	8.21×10^{-6}
IPN-3	3.75	8.5×10^4	2.72×10^{-5}
IPN-5	5.00	1.2×10^5	3.51×10^{-5}
IPN-7	7.50	1.6×10^5	4.34×10^{-5}
IPN-10	10.00	2.0×10^5	5.18×10^{-5}
NiPAAm ^b	0.00	8.8×10^4	2.76×10^{-5}

^a NiPAAm network obtained by UV polymerization in DMAc.

^b NiPAAm network obtained by temperature-initiated polymerization in dioxane.

showed a significantly higher compression modulus and thus greater mechanical strength. The increased modulus was directly related to the swelling levels of semi-IPNs as demonstrated in Figure 4, which shows the changes of the compression modulus and polymer volume fraction in the hydrated networks as a function of Biomer weight percent.

For the purpose of comparison, Table 2 also contains data for the cross-linked poly(NiPAAm) obtained by temperature-initiated polymerization in dioxane. The effective cross-linking density which was obtained for that network, with a cross-linking efficiency of 31%, corresponds to the values reported in the literature for cross-

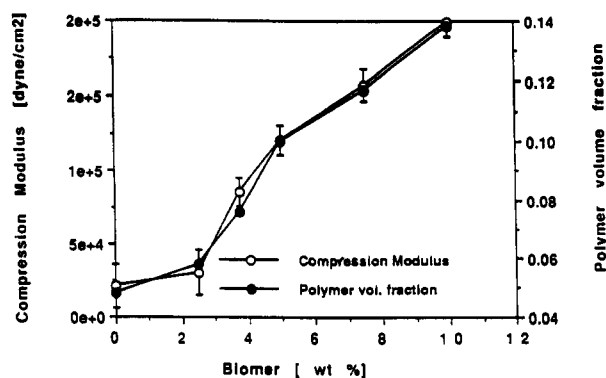


Figure 4. Effect of swelling level (expressed as polymer volume fraction in the swollen gel) and Biomer content on the compression modulus of cross-linked poly(NiPAAm) and NiPAAm/Biomer semi-IPNs. Error bars represent the standard deviation for $n = 3$.

Table 3. Glass Transition Temperatures, T_g , of Cross-Linked Poly(NiPAAm) and NiPAAm/Biomer Semi-IPNs

network	T_g (°C)	network	T_g (°C)
NiPAAm	112	IPN-7	86
IPN-2	108	IPN-10	85
IPN-3	93	Biomer ^a	-69
IPN-5	86		

^a solvent-cast film.

linked poly(NiPAAm) networks synthesized with 1 mol % of EGDMA.^{5,26} Different cross-linking efficiencies of NiPAAm networks synthesized under different conditions point to the strong effect of synthetic conditions on the properties of these networks. Due to the lower cross-linking efficiency, and in consequence lower cross-linking density, the NiPAAm network obtained by UV polymerization showed higher swelling levels than other NiPAAm networks cross-linked with 1 mol % of EGDMA, reported in the literature.⁵

Glass Transition Temperatures. In studies of IPNs, measurements of the glass transition temperatures (T_g s) are useful for structure determination. Two T_g s are indicative of phase-separated structure, with the T_g s of individual components often shifted toward each other indicating partial mixing of the networks.²⁷ T_g s of poly(NiPAAm), Biomer, and all semi-IPNs in the dry state are summarized in Table 3. The T_g determined for Biomer, -69 °C, corresponded to the literature values for elastomeric poly(etherurethanes).²⁰ Cross-linked poly(NiPAAm) (dry state) had a T_g of 112 °C, typical for acrylic polymers. For semi-IPNs, one transition, below 100 °C, was observed for all compositions. This transition corresponded to the T_g of cross-linked poly(NiPAAm). Due to the small Biomer content in semi-IPNs, a T_g of polyurethane glass transition was not detectable. For all semi-IPNs, small shifts of the T_g of cross-linked poly(NiPAAm) toward lower temperatures were observed with increasing Biomer content in the network. The shifts of T_g s suggested that partial mixing of the components occurred in the NiPAAm/Biomer semi-IPNs. As indicated by T_g s, the dehydrated Biomer/NiPAAm semi-IPNs were hard and glassy at room temperatures. After equilibration in water at room temperature all networks were soft and highly swollen.

Equilibrium Swelling. Equilibrium swelling studies of NiPAAm/Biomer semi-IPNs in isotonic PBS were conducted in a 5–40 °C temperature range, with the results presented in the Figure 5. Cross-linked poly(NiPAAm) dramatically deswells around 32 °C, i.e., at the temperature corresponding to the LCST of the linear NiPAAm

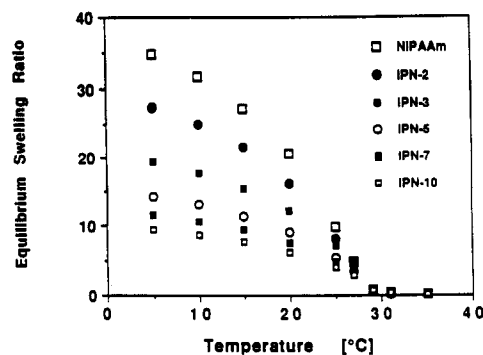


Figure 5. Equilibrium swelling of cross-linked poly(NiPAAm) and NiPAAm/Biomer semi-IPNs in isotonic PBS: effect of temperature and IPN composition. Error bars represent the standard deviation for $n = 3$.

polymer.² LCST results from the temperature dependence of polymer–polymer and polymer–water interactions such as hydrogen-bonding and hydrophobic interactions. The presence of Biomer did not change the gel collapse temperature (GCT) of semi-IPNs; however, the thermosensitivity and swelling levels at low temperature range (below the GCT) were decreased. The decreased swelling levels are related to the hydrophobicity of Biomer and the higher apparent effective cross-linking densities of semi-IPNs (see Table 2). The incorporation of a hydrophobic polymer into the thermosensitive gel by the semi-IPN formation may therefore be used to control the thermosensitivity and swelling levels of TSH without affecting the GCT. Similar results were reported with the thermosensitive IPNs composed of NiPAAm and poly(tetramethylene glycol) (PTMEG) cross-linked with a triisocyanate compound.¹¹ In these networks, the swelling and the thermosensitivity decreased due to hydrophobicity of the PTMEG network and also as a result of the physical restriction of the NiPAAm network.

Deswelling Kinetics. Cross-linked poly(NiPAAm) equilibrated in aqueous solutions at a temperature below the LCST, i.e., 32 °C, is highly swollen. If this swollen gel is immersed in a solution at a temperature above the LCST, collapse of the polymer network results in a deswelling process and a significant decrease of the gel volume. In the absence of a kinetic barrier, deswelling should occur very rapidly. However, in the presence of any kinetic barrier (e.g., a dense surface layer), the process of reaching the equilibrium swelling level above the LCST may be significantly retarded. The time scale of a deswelling process is important because it directly affects the release profiles of solutes imbedded in the swollen gel.

An interesting phenomenon observed during deswelling of cross-linked poly(NiPAAm) has been described.¹¹ When the temperature was increased through the gel collapse point, formation of water pockets on the gel surface was observed by optical microscopy. This phenomenon was explained as follows: when the swollen poly(NiPAAm) gel is exposed to a temperature above its collapse point, the outermost layer of the gel deswells rapidly forming a dense surface, described as a skin-type barrier. If the deswollen surface layer is dense enough to retard the efflux of water, the water pockets will appear on the gel surface. This skin formation process was shown to be a function of the gel composition and the temperature gradient between the low- and high-temperature medium.²³ NiPAAm gel containing a hydrophobic comonomer, butyl methacrylate, demonstrated enhanced skin formation, while gel containing a hydrophilic comonomer, acrylic acid, did not form a surface skin layer.

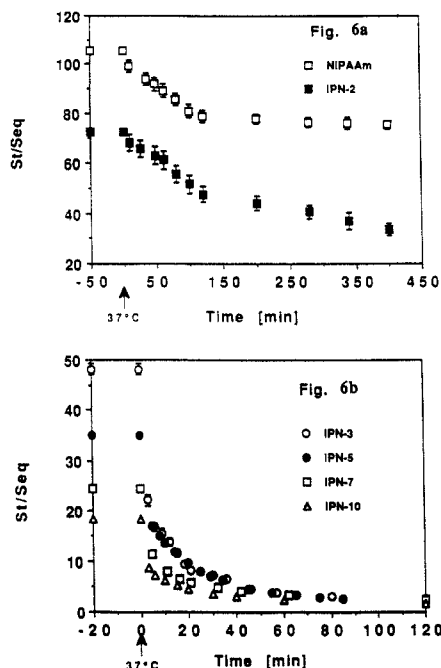


Figure 6. Deswelling kinetics of cross-linked poly(NiPAAm) and NiPAAm/Biomer semi-IPNs equilibrated at 23 °C and immersed at 37 °C at time 0. (a) Cross-linked poly(NiPAAm) and IPN-2. Error bars represent the standard deviation for $n = 4$. (b) IPN-3, -5, -7, and -10. Error bars represent the standard deviation for $n = 3$; for some data points error bars are smaller than the plot symbols.

In this study the effect of Biomer content on the deswelling kinetics of NiPAAm/Biomer semi-IPNs was examined. The deswelling kinetics profiles of gels equilibrated at 23 °C (room temperature) and at time 0, immersed directly in PBS pre-equilibrated at 37 °C, are presented in Figure 6. Results are plotted as the fractional approach to equilibrium, S_t/S_{eq} , where S_t denotes the swelling ratio of a gel at time t and S_{eq} denotes the equilibrium swelling ratio at 37 °C. The deswelling profile of IPN-2 was very similar to that of cross-linked poly(NiPAAm) (Figure 6a). In both cases, numerous water pockets were observed on the gel surface after immersion of the swollen gel at 37 °C, and a very slow deswelling process followed. It is interesting to note that the equilibrium swelling ratio of cross-linked poly(NiPAAm) equilibrated at 23 °C is more than 100 times higher than its equilibrium swelling ratio at 37 °C (Figure 6a; $S_t/S_{eq} = 107$ at time 0). It took more than 24 h for the gels to reach the equilibrium at 37 °C ($S_t/S_{eq} = 1$).

In contrast, IPN-3, -5, -7 showed relatively fast deswelling and all reached equilibrium within 1 h (Figure 6b). Formation of water pockets on the gel surface was not observed for IPN-3, -5, -7 and -10, which suggested that Biomer interfered with the skin formation process. Such results were not anticipated because Biomer, a hydrophobic component, was expected to enhance the skin formation process and slow down the deswelling of NiPAAm/Biomer semi-IPNs. Enhancement of the skin formation process by hydrophobic components was observed for NiPAAm gels containing butyl methacrylate and for NiPAAm IPNs containing cross-linked poly(tetramethylene glycol).¹¹

However, the results described by Dong et al.²⁸ point to yet another mechanism of fast deswelling of NiPAAm gels containing hydrophobic components. The authors synthesized NiPAAm gel containing vinyl-terminated poly(dimethylsiloxane) (PDMS) as a macrocross-linker. In these gels, phase separation of the components resulted

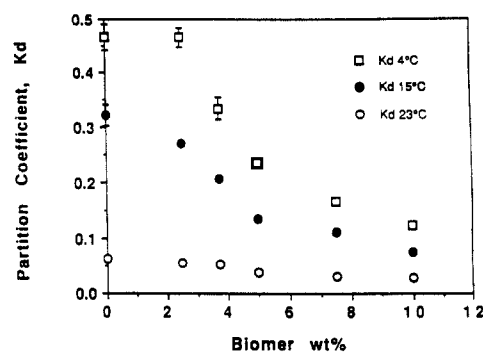


Figure 7. Partitioning of heparin into cross-linked poly(NiPAAm) and NiPAAm/Biomer semi-IPNs: effect of temperature and polymer composition. Error bars represent the standard deviation for $n = 3$.

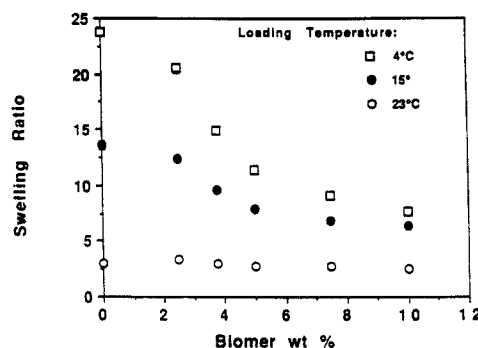


Figure 8. Equilibrium swelling of NiPAAm/Biomer semi-IPNs in loading solutions of heparin: effect of temperature and polymer composition. Error bars represent the standard deviation for $n = 3$.

in very fast deswelling rates with no skin formation. NiPAAm/Biomer semi-IPNs also showed extensive phase separation in the hydrated state as confirmed by their opacity increasing with Biomer content. The fast deswelling rates of NiPAAm/Biomer semi-IPNs may therefore result from the phase separation of the network components on the molecular level.

Heparin Loading. Factors affecting the partitioning of solutes into a swollen polymeric matrix are important issues in drug delivery and biomaterials research. In this study, the partitioning of heparin into the cross-linked poly(NiPAAm) and NiPAAm/Biomer semi-IPNs was examined. Heparin is a negatively charged glycosaminoglycan macromolecule, containing carboxylate and sulfate groups, ranging in molecular weight from 3000 to 35 000. Heparin used in these studies had a weight-average molecular weight of 6000 and a polydispersity index of $0.24 \pm 10\%$, determined using dynamic LLS. The partition coefficients of heparin in the networks were determined at 4, 15, and 23 °C (room temperature). Changes in partition coefficients, K_d , as a function of loading temperature and Biomer content in the networks are presented in Figure 7. It was observed that changes in the Biomer content significantly affected heparin loading at 4 and 15 °C, with minimal effect at room temperature. These results might be explained by studying the effect of Biomer content on the equilibrium swelling of semi-IPNs in a heparin-loading solution (Figure 8). At room temperature, the changes in swelling degree with increasing Biomer content are negligible. However, at 4 and 15 °C the equilibrium swelling in a heparin solution decreased significantly as the Biomer content in the IPN increased.

The partition coefficient, K_d , which decreased with increasing loading temperature and Biomer content in

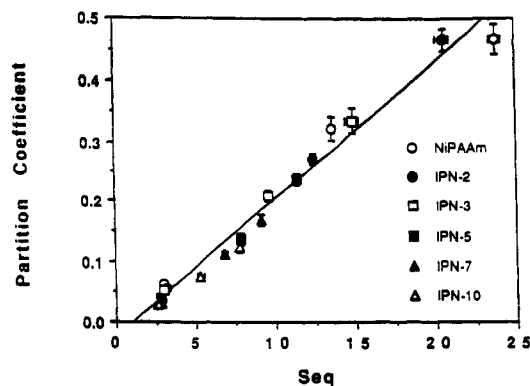


Figure 9. Linear relationship between heparin partition coefficients and equilibrium swelling levels in heparin loading solutions. Plot constructed from data for all networks at 4, 15, and 23 °C. Error bars represent the standard deviation for $n = 3$. Correlation coefficient = 0.977, as determined by a least-squares method.

semi-IPNs, was found to be linear function of S_{eq} , the equilibrium swelling ratio in heparin loading solutions (Figure 9). The same relationship was observed for all IPN compositions, as well as for the cross-linked poly(NiPAAm), even though changes in K_d associated with temperature were very different for each IPN. The highest value of the partition coefficient, and hence the highest loading levels, was obtained for the NiPAAm network at 4 °C. However, even in this case the partition coefficient was about 0.5 and did not approach 1.

The value of the partition coefficient, defined as the ratio of solute equilibrium concentration in the gel to that in the solution, depends upon a number of contributions, including electrical potential, specific gel-solute interactions, solute size, and conformational effects. In the case of hydrophilic solute partitioning into neutral gel, the size exclusion is often assumed as the dominant factor. The partition coefficient value should approach 1 at high swelling levels, where the screening effect of the network is negligible, and fall toward zero in the collapsed gel. The screening effect of the polymer network can be evaluated by comparing the diameters of the solutes with the mesh size of the network. The mesh size of similar NiPAAm networks was determined to be 27.9 nm at 20 °C and 19.5 nm at 25 °C.⁵ These values are significantly higher than the 3.2-nm radius of gyration of heparin (weight-average molecular weight 6000), estimated with dynamic LLS.²⁹ The mesh size of the cross-linked poly(NiPAAm) obtained in our experiments is expected to be even larger due to the lower cross-linking density, and hence higher equilibrium swelling levels of the UV cross-linked poly(NiPAAm) (see Table 2 for cross-linking density and Figure 4 for equilibrium swelling). Thus, the size-exclusion effect should not be significant in the case of partitioning of heparin into the highly swollen cross-linked poly(NiPAAm) at 4 °C. Since heparin is a charged macromolecule, the osmotic effects should be considered in the description of partitioning. It was observed previously that gel swelling levels in a heparin solution decreased with increasing heparin concentration.²³ The osmotic effects were implicated in the explanation of this observation.

In order to check whether heparin was actually diffusing into the core of the disks and was homogeneously distributed within the matrix, loading experiments were performed with disks of different thicknesses. The results showed that comparable loading percentages ($\pm 2\%$) were obtained with disks having the same radius, but differing by a factor of 2 in thickness. Therefore, it can be concluded that disks were loaded homogeneously.

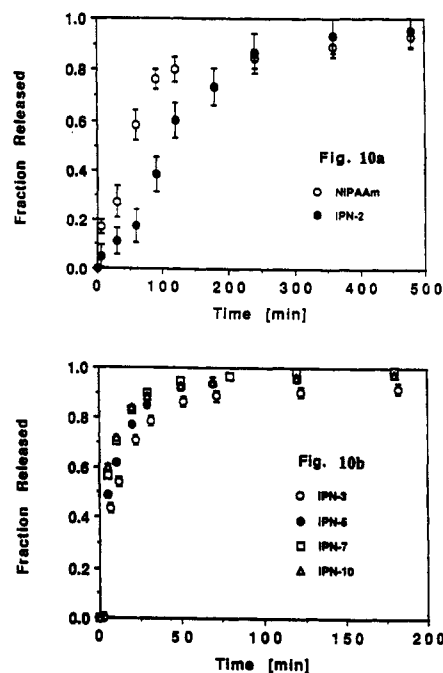


Figure 10. Heparin release from NiPAAm/Biomer semi-IPNs. (a) Release from cross-linked poly(NiPAAm) and IPN-2. (b) Release from IPN-3, -5, -7, and -10. Error bars represent the standard deviation for $n = 3$.

Heparin Release. As discussed in the previous section, high swelling levels of the synthesized NiPAAm/Biomer semi-IPNs at temperatures below their collapse point allowed for the absorption of significant amounts of heparin into the swollen gel matrix. In this section we discuss the release kinetics of the absorbed macromolecule at 37 °C, i.e., above the gel collapse point.

The release of solutes from thermosensitive polymers, at temperatures above their collapse point, involves an additional driving force for the macromolecule to leave the gel matrix, namely, the squeezing action of the collapsing polymer network. In the case of NiPAAm/Biomer semi-IPNs, significant volume changes were observed upon the immersion of swollen gel disks at 37 °C, i.e., at the temperature above the gel collapse point (see Figure 6). It was expected that the release kinetics of macromolecules imbibed in the swollen polymer network will be strongly affected by the gel deswelling process.

Cross-linked poly(NiPAAm) and NiPAAm/Biomer semi-IPNs loaded with heparin at room temperature were immersed directly into a 37 °C release medium. No drying procedure was applied to swollen and loaded gel disks. The observed heparin release profiles are presented in Figure 10 as a fractional release of solute. Cross-linked poly(NiPAAm) and IPN-2 (Figure 10a) demonstrated slower release rates than other IPNs (Figure 10b). These results correlated with the slow deswelling process observed for the cross-linked poly(NiPAAm) and IPN-2 due to the formation of a skin layer on the gel surface. Skin formation, confirmed by the presence of water pockets, was also observed in the heparin release process. The skin acted as a rate-controlling barrier for macromolecular diffusion, and lower heparin release rates from cross-linked poly(NiPAAm) and IPN-2 were observed. In the case of IPN-3, -5, -7, and -10, the rapid release (squeezing) of the macromolecule from the collapsing gel matrix was completed within 1 h. The skin formation was not observed for these networks during the release process. Higher release rates of heparin from IPN-3, -5, -7, and -10 were in agreement with the rapid deswelling of these networks. For cross-linked poly(NiPAAm) and NiPAAm/Biomer

semi-IPNs, the network deswelling kinetics was a dominant factor in the release of the macromolecule.

The release of heparin from the skin-forming cross-linked poly(NiPAAm) and IPN-2 is in contrast with the results reported for NiPAAm gels containing hydrophobic components,¹¹ where the skin formation resulted in complete blocking of solute flux at temperatures above the GCT. However, results similar to ours were reported by Afrassiabi et al.,³⁰ describing the release of vitamin B12 from highly swollen, loosely cross-linked NiPAAm gels with the surface skin layer forming at temperatures above the gel collapse point. The initial rapid release of vitamin B12 was followed by a slow release phase in which the collapsed outer gel layer acted as a rate-controlling barrier.

Based on the results obtained in this study and other results described in the literature, the following four mechanisms of solute release from swollen NiPAAm TSH should be considered:

1. *An on-off thermocontrol of solute release with temperature modulation*¹¹ (involves skin formation). This profile is observed only when a sufficiently thick skin forms during the off phase due to the immediate shrinking of the outer gel layer, blocking the outflow of bulk water and solutes from the gel. The complete impermeability of the solute through the skin layer in the off phase is possible only with a small gradient of equilibrium swelling degree between the on and off phases.

2. *Initial rapid release followed by a slower release phase* (involves skin formation). This profile is observed when the initial rapid shrinking of the highly swollen gel is followed by a slower deswelling process. This mechanism requires a significant gradient of the equilibrium swelling degree, ΔS_{eq} , between the loading and release medium. The high ΔS_{eq} creates a hydrostatic pressure that causes the expansion of water pockets, and only a thin skin forms on the gel surface. This release mechanism was observed in this study for heparin released from the NiPAAm network and IPN-2, for vitamin B12 released from loosely cross-linked NiPAAm gels,³⁰ and for heparin released from NiPAAm/BMA gel at 37 °C after loading at 1 and 15 °C.²³

3. *Rapid squeezing of the solute imbibed in the gel matrix* (no skin formation, low gel equilibrium swelling at 37 °C). This profile is observed for gels containing components that deter skin formation. Fast deswelling of a gel results in a rapid squeezing of the solute from the gel matrix. Low molecular weight solutes are released (squeezed out) completely, while high molecular weight compounds may remain partially trapped in the collapsed gel matrix. This mechanism was observed in these studies for heparin releasing from IPN-3, -5, -7, and -10.

4. *Initial rapid release (squeezing) followed by a slow release phase* (no skin formation, higher gel equilibrium swelling at 37 °C). This profile is observed for NiPAAm gels containing hydrophilic components that do not exhibit skin formation and have relatively high equilibrium swelling at 37 °C. In this case, the initial fast squeezing is followed by a slow release from the collapsed network. This kind of release profile was observed for heparin releasing from NiPAAm gel containing 2 mol % of acrylic acid (AAc). Prolonged release of heparin from the collapsed NiPAAm/AAc gel was possible due to the relatively high water content (about 50%) in the gel at 37 °C.²³

In summary, the release of solutes from swollen TSH gels is determined by their deswelling kinetics, which in turn depends on the gel composition, cross-linking density,

and synthetic conditions, as well as on the temperature gradient between the loading and release medium.

Conclusions

Novel thermosensitive NiPAAm/Biomer semi-IPNs were synthesized. The networks exhibited superior mechanical properties when compared with the cross-linked poly(NiPAAm) due to the increased apparent effective cross-linking densities and lower swelling levels. Equilibrium swelling studies showed that modification of NiPAAm gel with Biomer via a semi-IPN formation did not affect the gel collapse point but resulted in decreased thermosensitivity and lower swelling levels. Deswelling kinetics of NiPAAm/Biomer semi-IPNs was markedly affected by the Biomer content. Only the semi-IPN containing 2.5 wt % Biomer exhibited a deswelling rate comparable to deswelling of cross-linked poly(NiPAAm). Semi-IPNs containing more than 2.5 wt % of Biomer showed much faster deswelling rates as a result of Biomer preventing formation of a skin-type layer on the gel surface. It was hypothesized that the fast deswelling rates of NiPAAm/Biomer semi-IPNs resulted from the phase separation of the network components.

Heparin partition coefficients into the cross-linked poly(NiPAAm) and all NiPAAm/Biomer semi-IPNs loaded at different temperatures were found to be a linear function of the network equilibrium swelling in drug-loading solutions. The amount of heparin partitioning into the swollen polymer matrix increased with decreasing loading temperature and Biomer content. Heparin release profiles from cross-linked poly(NiPAAm) and NiPAAm/Biomer semi-IPNs correlated with the deswelling kinetics of the networks. Heparin release from cross-linked poly(NiPAAm) and IPN-2 was controlled by the surface skin layer, which slowed down heparin diffusion from the swollen polymer network. The semi-IPNs containing more than 2.5 wt % of Biomer demonstrated a faster release rate. In this case, the release rate was controlled by the kinetics of a gel collapse, that resulted in fast "squeezing" of heparin from the gel.

The mechanism of solute release from swollen TSH gels is determined by the gel deswelling kinetics, which in turn depends on the gel composition, cross-linking density, and synthetic conditions, as well as on the temperature gradient between the loading and release medium.

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