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ORIGINAL PAPER

pH- and thermo-sensitive semi-IPN hydrogels composed of chitosan, *N*-isopropylacrylamide, and poly(ethylene glycol)-*co*-poly(ε-caprolactone) macromer for drug delivery

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Abstract In this study, semi-IPN chitosan/poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogels have been prepared via in situ UV-photo-crosslinking of *N*-isopropylacrylamide monomer using poly(ethylene glycol)-*co*-poly(ε-caprolactone) (PEG-*co*-PCL) macromer as a crosslinker in the presence of chitosan. Swelling properties of the resultant hydrogels were studied by investigating pH- and temperature dependence of equilibrium swelling ratio and oscillatory swelling–deswelling kinetics. It was found that semi-IPN hydrogels responded to both temperature and pH changes, and such stimuli-responsiveness was rapidly reversible. The rheological measurements demonstrated that the incorporation of chitosan greatly improved the mechanical strength of the hydrogels prepared. The release profiles of bovine serum albumin (BSA) from the hydrogels were also evaluated. The results showed that the release rate of BSA was higher in pH 2.0 buffer solution than in pH 7.4 buffer solution at 37 °C. Such double-sensitive hydrogels have the potential to use as smart carriers for drug delivery systems.

Keywords Chitosan · Semi-IPN hydrogels · Photo-crosslinking · Stimuli-response · Drug delivery

Introduction

During the past decade, environmentally intelligent polymeric hydrogels have attracted special attention due to their distinguished properties and potential applications in biomedical fields [1–3]. Particularly, thermo-/pH-sensitive hydrogels are of great interest in drug delivery because the release rate of drugs embedded into

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these hydrogels could be regulated by altering the local temperature/pH [4, 5]. Among the thermo-sensitive hydrogels, poly(*N*-isopropylacrylamide) (PNIPAAm) has been extensively studied for pharmaceutical applications due to its unique thermo-gelation properties, exhibiting a lower critical solution temperature (LCST) at around 32 °C [6]. To prepare thermo-sensitive PNIPAAm hydrogels with pH sensitivity, an approach to copolymerize NIPAAm monomer with other comonomer bearing weakly acidic groups such as acrylic acid has been widely evaluated [7, 8]. However, the temperature sensitivity of copolymerized hydrogels might be weakened due to the incorporation of pH-sensitive components [9, 10].

In recent years, semi-interpenetrating polymer network (semi-IPN) hydrogels containing two more different networks have attracted great research interest. Semi-IPN structures have been extensively utilized to improve the mechanical strength and the biocompatibility of the resultant hydrogels [11, 12]. It is also believed that each of polymers in the semi-IPN structures can keep its individual properties, thereby such structures can be used to introduce other properties by adjusting the polymer networks [13]. Many researchers have synthesized semi-IPN hydrogels derived from PNIPAAm to improve its properties or introduce other properties into PNIPAAm networks without weakening its thermo-sensitive property. Muniz et al. introduced PNIPAAm into crosslinked poly(acrylamide) (PAAm) network to obtain semi-IPN hydrogels, and these hydrogels exhibited good mechanical properties and kept the thermo-sensitive property of PNIPAAm [14]. It was also reported that poly(vinyl alcohol) (PVA)/PNIPAAm semi-hydrogels showed a fast response rate to temperature changes compared to conventional PNIPAAm gels [15, 16]. Alvarez-Lorenzo et al. first prepared chitosan-poly(*N*-isopropylacrylamide) semi-IPN hydrogel, then fabricated temperature-responsive and pH-sensitive IPN hydrogel via the post-cross-linking of chitosan [17]. However, to prepare above-mentioned semi-IPN hydrogels, *N*,*N*-methylenebisacrylamide (MBAAM) is often employed as a crosslinker, such hydrogels produced have a limitation in clinical applications due to their partial or non-biodegradability [18].

Photo-crosslinked hydrogels under UV or visible light have been investigated widely as drug delivery systems, because the reaction conditions are very mild and they can be formed in situ at a specific site at a rapid polymerization rate. Of these hydrogels, hydrogels based on PEG bearing α-hydroxyl acid block copolymers terminated with acrylate groups have been evaluated as delivery vehicles of bioactive macromolecular drugs and cells due to their biodegradability and biocompatibility [19, 20]. In our previous work, we synthesized biodegradable thermo- and pH-sensitive poly[(*N*-isopropylacrylamide)-*co*-(methacrylic acid)] hydrogels using a biodegradable PEG-*co*-PCL macromer as a crosslinker [21]. In this study, a kind of semi-IPN hydrogels was synthesized by physically incorporating chitosan into crosslinked PNIPAAm network using PEG-*co*-PCL macromer as a crosslinker under UV irradiation. Chitosan is a kind of abundant and naturally occurring hydrophilic cationic polysaccharide derived from chitin, which has been widely used for the controlled delivery of polypeptide and proteins due to its bioactivity, biocompatibility, biodegradability, and mechanical properties [22–24]. The hydrogels containing chitosan as semi-IPN structure could exhibit pH-sensitive behavior due to the amino groups along chitosan chains. In this study, the swelling

response of the semi-IPN hydrogels to pH and temperature was investigated, the mechanical properties of the hydrogels and the release profile of BSA in situ embedded into the hydrogels were also evaluated.

Experimental

Materials

PEG ($M_n = 6000$, Japan) was dehydrated by azeotropic distillation in toluene. ε-Caprolactone (99%, Aldrich, USA) was dried over CaH_2 for 48 h and distilled under vacuum just before use. *N*-isopropylacrylamide (NIPAAm) (97%, Aldrich, USA) was purified by recrystallization from a 65:35(v/v) mixture of hexane and benzene and dried under vacuum for 2 days. Glycidyl methacrylate (GMA) (97%, Aldrich, USA), 4-dimethylaminopyridine (DMAP) (99%, TCI, Japan), 1-vinyl-2-pyrrolidone (NVP) (97%, Fluka, USA), stannous 2-ethyl hexanoate (95%, Sigma, USA), and the photoinitiator, 2,2-dimethoxy-2-phenylacetophenone (DMPA) (99%, Acros, USA), were used as received. Chitosan ($M_w = 4.5 \times 10^5$, degree of deacetylation = 90%) was purchased from RuJi Biotech Development Co., Ltd (Shanghai, China). The other chemicals used were of analytical grade and used without further purification.

Synthesis of PEG-*co*-PCL macromer

PEG-*co*-PCL macromer was synthesized as follows: First, PEG-*co*-PCL block copolymer was synthesized via the ring-opening polymerization of ε-caprolactone initiated by PEG ($M_n = 6000$) at a molar ratio of 6:1 ([CL]/[PEG]) using stannous 2-ethyl hexanoate as a catalyst. For the second step of synthesis of PEG-*co*-PCL macromer, 10 g of the above-mentioned block copolymer was dissolved in 100 mL of dichloromethane in a 250-mL round-bottomed flask, and 1.28 g of GMA and 0.37 g of DMAP were added to the solution. The reaction mixture was stirred at room temperature for 48 h. The crude product was precipitated in an excess of anhydrous ethyl ether. The product was further purified by dissolving it in dichloromethane, precipitated in anhydrous ethyl ether twice and then dried under vacuum at room temperature for 2 days.

Preparation of the semi-IPN hydrogels

To synthesize semi-IPN hydrogels, NIPAAm, PEG-*co*-PCL macromer, and chitosan at different ratios were dissolved in 2 wt% HAc solution (4.5 g) at room temperature. Then the photoinitiator solution of DMPA in NVP (300 mg/mL) was added to the mixture (1 wt% DMPA to the total amount of the macromer and NIPAAm monomer), the resulting solution was homogeneously mixed and added to a Teflon model. Following this, the mixture was exposed to 365 nm LWUV lamp of 16 W (ZF-7A type, Shanghai Jihui Scientific Instrumental Co. Ltd) for 10 min to ensure the enough copolymerization of NIPAAm monomer with the macromer. The

distance between the reaction mixture and the light source is kept 2 cm. As a control, the copolymerized hydrogel without adding chitosan was synthesized at the same conditions.

The photo-crosslinked hydrogel was cut into disks (10 mm in diameter and 3 mm in thickness). To extract the residual unreacted monomers and other impurities in the obtained hydrogel samples, the samples were immersed in distilled water for 3 days at 20 °C, and the distilled water was refreshed every 12 h. Later, the samples were dried at room temperature for 2 days and then dried at 60 °C under reduced pressure for another 2 days. The feed compositions of the hydrogels in this study are listed in Table 1.

Instrumental analysis and measurements

FT-IR spectra of dry gels were carried out using KBr pellets on a NICOLET-670IR spectrometer, and ^{1}H NMR spectrum of the PEG-*co*-PCL macromer was recorded on a Bruker AV400 spectrometer at 400 MHz using $CDCl_3$ as the solvent. The gravimetric method was employed to measure the equilibrium swelling ratios (ESR) of the hydrogels. The equilibrium swelling studies were performed in buffer solutions of different pH (2.0 and 7.0) at different temperatures (from 10 to 55 °C) and in buffer solutions of different pH (from 2.0 to 8.0) at room temperature. The ionic strength of the buffer solutions was fixed at 0.1 mol/L. The hydrogels were immersed in buffer solution to reach a swollen equilibrium at each predetermined temperature or predetermined pH, and the equilibrated swollen hydrogels were weighed after excess surface water was removed carefully with moistened filter paper. All experiments were performed in triplicate for each of the samples, and the average value of three measurements was taken. The ESR was calculated according to the following equation:

$$\mathrm{ESR}\ (\%) = [(W_e - W_d)/W_d] \times 100,$$

where W_e and W_d denote the weights of the equilibrated swollen hydrogels and dried gels, respectively. The oscillatory swelling behavior was measured in buffer solution (pH 2.0, $I = 0.1$ mol/L) maintained at alternate temperatures of 20 and 45 °C, and in buffer solutions ($I = 0.1$ mol/L) with pH values between 2.0 and 7.4 at 20 °C. The weight of the hydrogels was measured at a predetermined time intervals at the temperatures and pH quoted. The rheological behaviors of the photo-crosslinked hydrogels were investigated by a strain-controlled AR2000ex rheometer

Table 1 Feed compositions for the preparation of hydrogels

Components						
Sample code	NIPAAm (g)	Chitosan (mg)	PEG-*co*-PCL macromer (g)	2 wt% HAc (g)	DMAP (mg)	Gel content (%)
PN-0	0.86	0	0.5	4.5	14	90.3
Semi-IPN1	0.86	92	0.5	4.5	14	92.5
Semi-IPN2	0.86	210	0.5	4.5	14	93.2

(TA Company, USA) with stainless-steel parallel plate geometry. Storage moduli and loss moduli were measured as a function of the frequency. Test samples were made to match the diameter of the parallel plate (40 mm). The gap between the parallel plates was adjusted to 1.2 mm. The measurement was performed through a dynamic frequency sweep test at 25 °C, and the strain was 0.1%, which is within the linear viscoelastic region, as determined by dynamic strain sweep experiments.

In vitro drug release from the hydrogels

Drug-loaded hydrogel was prepared using a similar method for the synthesis of semi-IPN hydrogels, in which 0.5 wt% BSA (relative to the total weight of formulation solution of the hydrogel) was added, the solution was homogeneously mixed before photopolymerization. After the photo-crosslinking, the disk-shaped gel was placed in a tube containing 12 mL of fresh PBS with horizontal shaking (pH 7.4 and 2.0, 37 °C). At predetermined time intervals, the solution was taken out and replaced in another 12 mL of fresh PBS. The concentration of BSA released was analyzed using UV spectrophotometer (UV-2550, SHIMADZU, Japan) at the maximum absorbance wavelength of BSA at 280 nm. All experiments were repeated three times, and the average value of three measurements was taken.

Results and discussion

Synthesis of the macromer and semi-IPN hydrogels

PEG-*co*-PCL macromer as a crosslinker was synthesized by the ring-opening polymerization of ε-caprolactone initiated by PEG and, subsequently, reacted with GMA. Figure 1 presents ^{1}H NMR spectrum of the macromer prepared. The small signals at 5.5–6.2 ppm belong to protons of the $–C(CH_3)=CH_2$ attached to the both ends of the block copolymer. The signals at 4.1, 2.3, 1.4, and 1.6 ppm are assigned to the CH_2 unit of PCL segments. The signal at 3.6 ppm results from the protons of CH_2CH_2 units of PEG segments. The signals at 4.1–4.3 ppm belong to the protons of CH_2CH_2O-PCL and $CH_2CH(OH)CH_2$ of GMA. The signal at 1.95 ppm is attributed to the protons of CH_3 group of GMA. The actual CL units in the macromer could be calculated from the signal intensity ratio of the $–CH_2CH_2–$ protons in the PEG block ($\sim$3.63 ppm) and the $–CH_2–$ ($\sim$4.10 ppm) of CL units and were found to be 5.4. These results indicate that the macromer as a crosslinker was successfully synthesized.

The hydrogels were prepared via in situ copolymerization of NIPAAm monomer with the macromer prepared as a crosslinker in the presence of the hydrophilic chitosan by UV irradiation technology. NIPAAm monomer with only one double bond will incorporate itself into the polymer backbone as PNIPAAm components along the polymethacrylate chains which are connected by PEG-*co*-PCL crosslinks in the network. The chitosan chains penetrate into PNIPAAm network crosslinked by PEG-*co*-PCL macromer. The synthetic route for semi-IPN hydrogel is illustrated in Fig. 2.

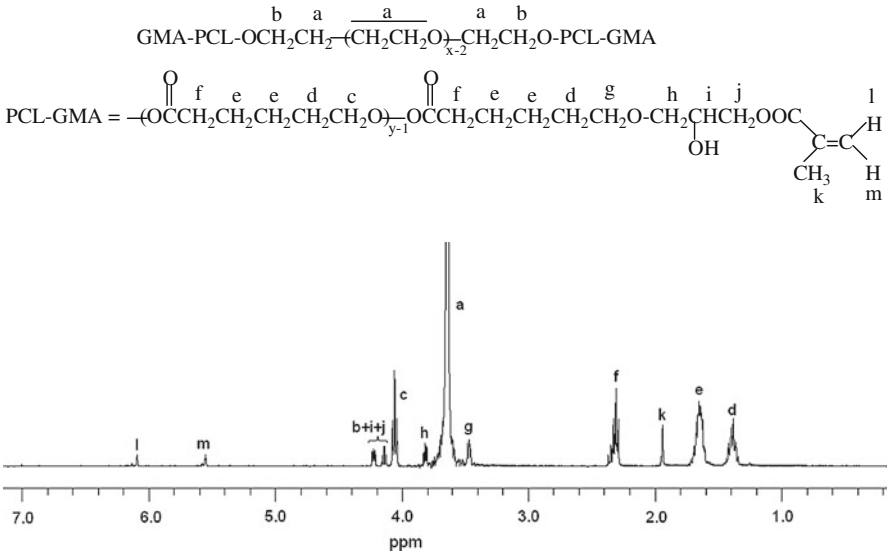

Fig. 1 ^{1}H NMR spectrum of the PEG-*co*-PCL macromer (in $CDCl_3$)

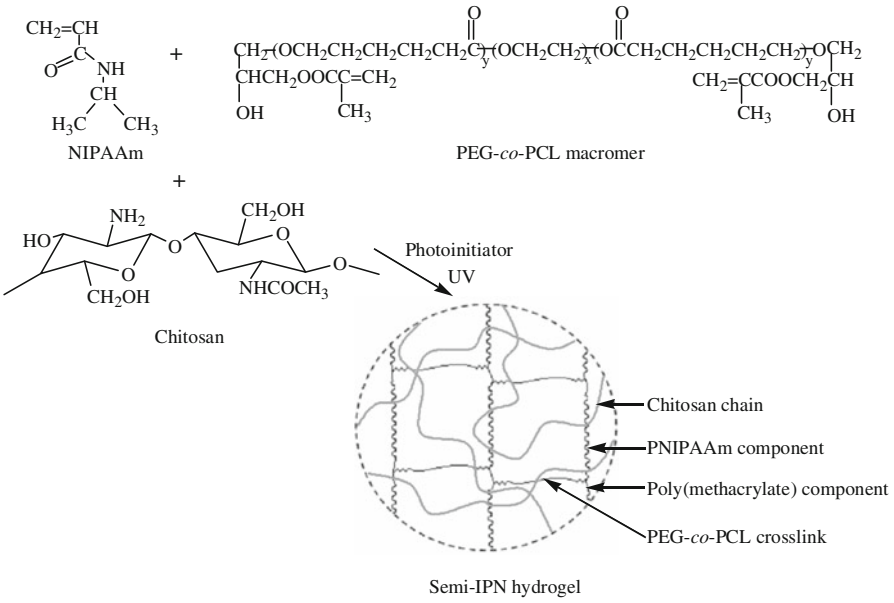

Fig. 2 Illustration for the preparation of chitosan/PNIPAAm semi-IPN hydrogel

FT-IR spectra of chitosan, the macromer, PN-0, and semi-IPNs samples are shown in Fig. 3. The spectra of PN-0 and semi-IPN samples exhibit a new band around 2970 cm^{-1}, which is assigned to C–N from PNIPAAm component. Two characteristic bands of the amide I and amide II of the amide group of PNIPAAm

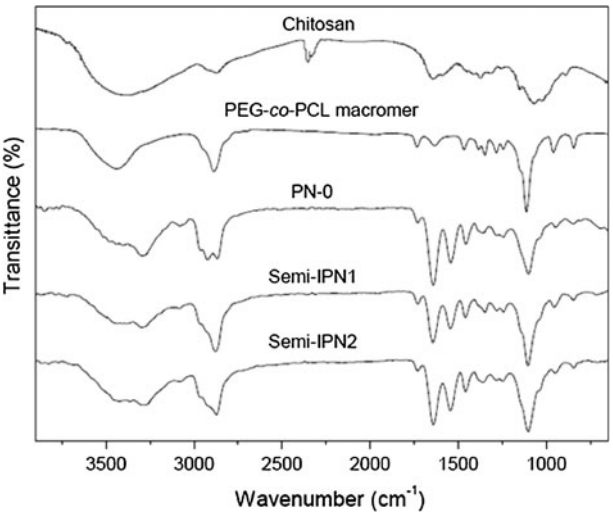

Fig. 3 FT-IR spectra of chitosan, the macromer, and PN-0, semi-IPN1, and semi-IPN2 dried gels

component and chitosan for the two semi-IPN samples are observed around 1639 and 1543 cm^{-1}, respectively [25]. The peaks at 1734 and 1109 cm^{-1} correspond to the carbonyl stretching mainly from PCL segments and –C–O–C– stretching from PEG segments, respectively.

pH sensitivity of the semi-IPN hydrogels

The effect of pH values on swelling ratio of the semi-IPN hydrogels was determined in buffer solutions in the pH range from 2.0 to 8.0 with a fixed ionic strength of 0.1 mol/L at 25 °C. As shown in Fig. 4, ESR of the hydrogels decreased obviously with the increasing pH of the buffer solution, and ESR values of the hydrogels exhibited a dramatic transition between pH 4.0 and 6.0. This phenomenon can be attributed to the ionization behavior of $–NH_2$ groups of chitosan in response to external pH changes. At lower pH value, most $–NH_2$ groups of chitosan are positively charged, and the hydrogen bonds between the inter- and intra-molecules are broken. Meanwhile, the electrostatic repulsion force between $–NH_3^+$ groups of chitosan chains leads to the expansion of the polymer network, these attract more water into the hydrogel network [26]. With the increase of pH values, $–NH_2$ groups were kept in the form of $–NH_2$, which may induce the formation of hydrogen bond between the inter- and intra-molecules, so the hydrogen bonds become dominant in the polymer network. Such enhancement of interactions between polymer chains causes the decrease in ESR of the hydrogels. In addition, when the content of the macromer and NIPAAm monomer was kept constant, it was also observed that ESR of semi-IPN hydrogels increased with the increasing chitosan content at lower pH value, which may be attributed to more $–NH_2$ groups to be positively charged leading to much more expanding hydrogel network. These results clearly indicated that the resultant semi-IPN hydrogels exhibited pH-sensitive characteristic.

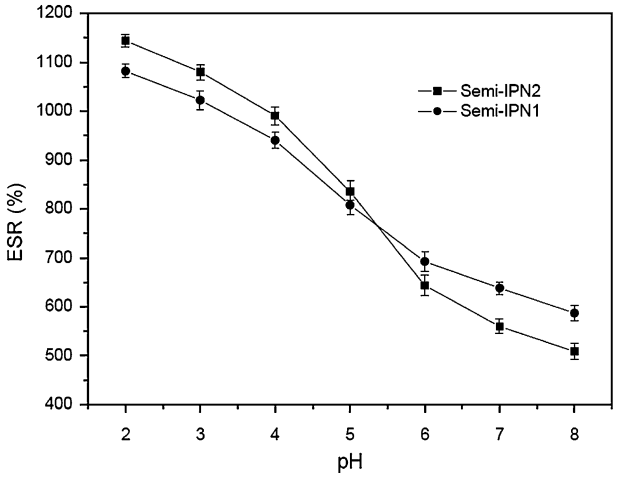

Fig. 4 Effect of pH of buffer solutions on ESR of the semi-IPN hydrogels at 25 °C

Figure 5 shows the pH-dependent swelling–deswelling behavior of the semi-IPN hydrogels between pH 2.0 and 7.4 in buffer solutions at 25 °C. The time interval for each step is set for 3 h, the semi-IPN hydrogels do not reach the equilibrium ratio during the period, as shown in Fig. 4. The experimental data still show that the semi-hydrogels display good reversibility to swell and shrink upon altering pH of the medium.

Temperature dependence of the semi-IPN hydrogels

The ESR of the hydrogels at pH 2.0 and 7.0 in buffer solutions as a function of temperature is depicted in Fig. 6. It was clearly observed that ESR of the hydrogels decreased with the increasing temperature both at pH 2.0 and 7.4, exhibiting a similar phase transition at around 33 °C. Semi-IPN has a interlocked structure of two polymer network without chemical bonds between them, so each of polymer networks keeps its individual properties. In the crosslinked PNIPAAm network, there exist hydrophilic and hydrophobic regions due to hydrophilic groups (–CONH–) and hydrophobic groups ($–CH(CH_3)_2$) from the NIPAAm monomer, respectively. At lower temperature (below the phase transition temperature of the hydrogels), the hydrogen bond interactions between the hydrophilic groups and surrounding water are dominant, hence, water penetrates into the polymer network leading to higher ESR. With the increase of the temperature, some of the hydrogen bonds are broken, and the hydrophobic interactions among the hydrophobic groups increase. As a result, the water molecules entrapped in the hydrogels are repulsed from the networks, resulting in the collapse of the hydrogel networks and decreasing the ESR of the hydrogels rapidly at the phase transition temperature [27].

As seen from Fig. 6, the ESR of the semi-IPN hydrogels increases with the increase of chitosan content at pH 2.0, but decreases at pH 7.0 at the same temperature. This might be attributed to the ionization of more $–NH_2$ groups from

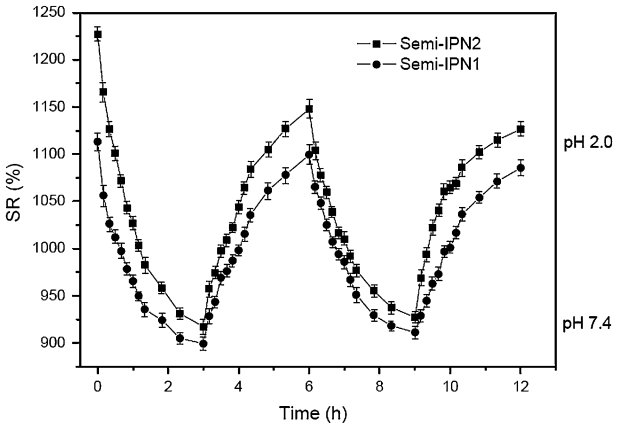

Fig. 5 Oscillatory swelling–deswelling behavior of the semi-IPN hydrogels in response to the alternating changes of pH in buffer solutions at 25 °C

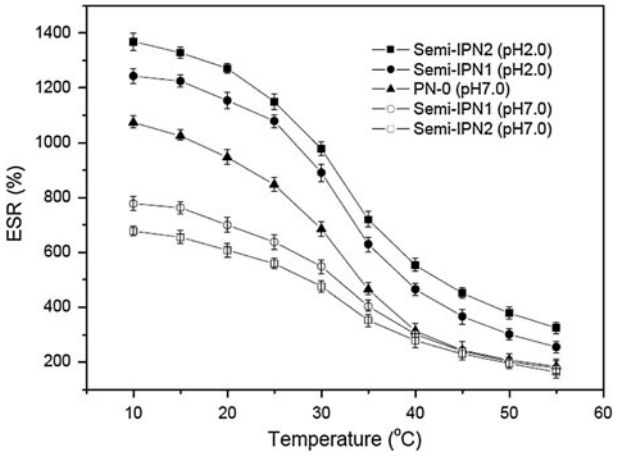

Fig. 6 Temperature dependence of the equilibrium swelling ratio for the semi-IPN hydrogels in buffer solutions of pH 2.0 and 7.0

chitosan leading to stronger electrostatic repulsion force at lower pH, which results in higher ESR of the semi-IPN hydrogels, whereas the formation of more hydrogen bonds induce much more compact polymer network at higher pH, decreasing the ERS of the semi-IPN hydrogels, as discussed above. It is also found that the temperature sensitivity of the semi-IPN hydrogels is stronger in acidic medium than that in basic medium.

Figure 7 exhibits the reversibility process of temperature response between 20 and 45 °C in buffer solution of pH 2.0. After the hydrogel achieves ESR at 20 °C, it begins to shrink dramatically when being immersed into a 45 °C buffer solution. During 30 min, the SR of the semi-IPN hydrogel decreases to approach its ESR. It is believed that chitosan in the semi-IPN hydrogels can act as a channel for water to

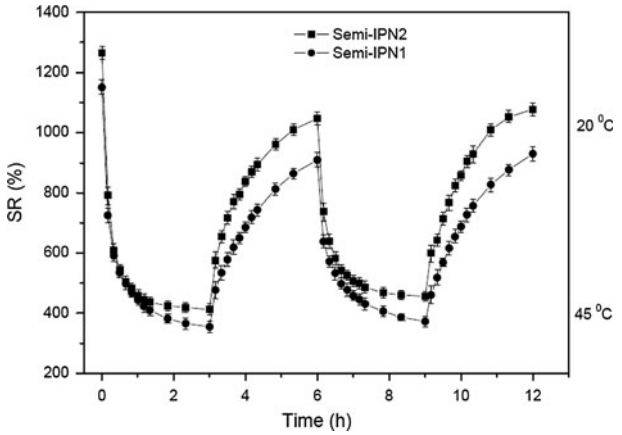

Fig. 7 Oscillatory swelling–deswelling behavior of the semi-IPN hydrogels in response to the temperature changes between 20 and 45 °C at pH 2.0

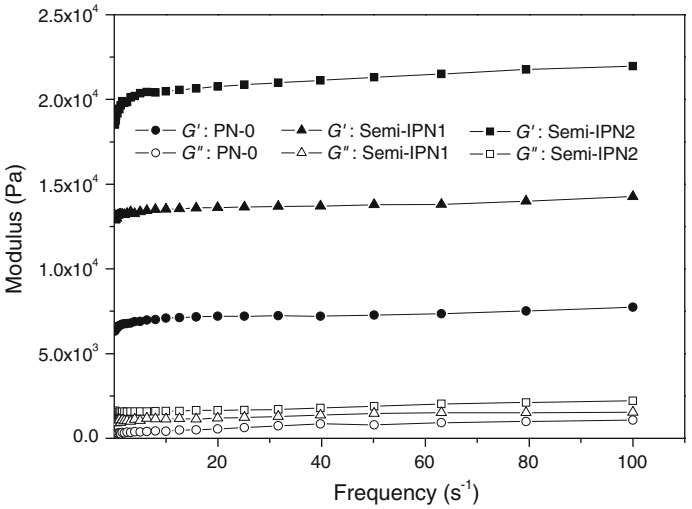

Fig. 8 Storage modulus (G') and loss modulus (G'') as a function of frequency for PN-0 and semi-IPN hydrogels at 25 °C

diffuse easily into or out of the polymer network. The repeated response of the semi-IPN hydrogels investigated to temperature changes is better than that to pH changes.

Rheological properties of the hydrogels

The dynamic mechanical rheology of PN-0 and semi-IPN hydrogels prepared was investigated by measuring storage modulus (G') and loss modulus (G'') as a function of frequency at 25 °C, as depicted in Fig. 8. For all the hydrogel samples investigated, the G' values are much greater than G'' values over the entire

frequency region, indicating that the photo-crosslinked hydrogel materials display a predominantly elastic solid-like behavior [28]. It is also observed that all semi-IPN hydrogel samples have greater storage moduli than that of PN-0 sample without chitosan, and that the storage modulus increases with the increase of the chitosan content incorporated into the hydrogels. These results demonstrate that the introduction of chitosan as the semi-IPN structure greatly improves the mechanical properties of the as-obtained semi-IPN hydrogels.

In vitro drug release from the hydrogels

Figure 9 shows the release profiles of BSA in situ embedded into different hydrogels in buffer solutions at pH 2.0 and 7.4 at 37 °C. The drug-loaded hydrogels showed an initial burst release for the first hour both in acidic and basic media, followed by slow release from the hydrogels. The initial burst release might be attributed to the rapid diffusion of BSA loaded close to the surface of the hydrogel. The release percentage of BSA is much higher at pH 2.0 than that at pH 7.4. This can be explained that the swelling ratio of the semi-IPN hydrogels is higher at pH 2.0 than that at pH 7.4, which enables BSA to diffuse through the swollen network into the external medium.

It can be also observed a tendency for an increase of BSA release with increasing content of chitosan in the semi-IPN hydrogels at pH 2.0 at 37 °C, but the release of BSA decreases at pH 7.4 at 37 °C. This could be also explained in terms of the swelling behavior of semi-IPN hydrogels. As can be seen from Fig. 6, the swelling ratio of the semi-IPN hydrogels increases with the increasing content of chitosan introduced at pH 2.0, while the swelling ratio decreases at the same hydrogel formulations at pH 7.4 and 37 °C. For the semi-IPN hydrogel matrix investigated, the release of BSA is regulated by the swelling behavior of the hydrogel. These

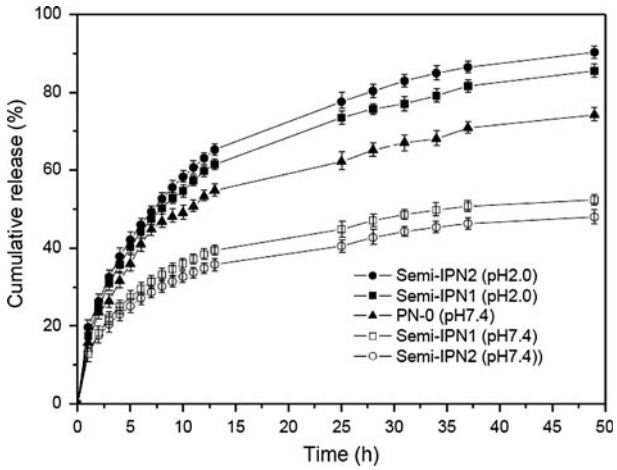

Fig. 9 The release profiles of BSA from the hydrogels in buffer solutions of pH 2.0 and 7.4 at 37 °C

results show that the BSA release from the hydrogels could be controlled by pH and chitosan content in the hydrogel.

Conclusions

In this study, the semi-IPN hydrogels composed of chitosan and PNIPAAm network crosslinked with PEG-*co*-PCL macromer were prepared by UV irradiation technology. Swelling measurement results showed that the resultant semi-IPN hydrogels displayed both pH and temperature sensitivity, and such responsive process exhibited good reversibility. The introduction of chitosan enhanced the mechanical strength of the semi-IPN hydrogels. In vitro release profiles of BSA from the hydrogels could be modulated by adjusting the pH and chitosan content in the hydrogel introduced. Such dual pH- and thermo-sensitive semi-IPN hydrogels could be expected to be useful for drug delivery systems.

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