Photo-Cross-Linked Biodegradable Thermo- and pH-Responsive Hydrogels for Controlled Drug Release

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ABSTRACT: A new strategy was developed to prepare thermo- and pH-sensitive hydrogels by the crosslinking of poly(*N*-isopropylacrylamide) with a biodegradable crosslinker derived from poly(L-glutamic acid). Hydrogels were fabricated by exposing aqueous solutions of precursor containing photoinitiator to UV light irradiation. The swelling behaviors of hydrogels at different temperatures, pHs, and ionic strengths were examined. The hydrogels shrank under acidic condition or at temperature above their collapse temperature and would swell in neutral or basic media or at lower temperature. These processes were reversible as the pH or temperature changed. All hydrogels exhibited no weight loss in the simulated gastric fluid but degraded rapidly in the simulated intestinal condition. Bovine serum albumin were used as a model protein drug and loaded into the hydrogels. The *in vitro* drug release experiment was carried out at different pH values and temperatures. The pH and temperature dependent release behaviors indicated the promising application of these materials as stimuli-responsive drug delivery vehicles. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 123: 2923–2932, 2012

Key words: stimuli-sensitive polymers; hydrogels; controlled release; biodegradable

INTRODUCTION

Stimuli-responsive polymers have been extensively investigated for decades because of their unique ability of mimicking basic response processes of living systems.[1–6] Recently, various materials based on these polymers were designed and applied in biomedical fields including drug delivery, tissue engineering, bioseparation, and biosensor.[7–10] Many polymers have been developed to respond to the external stimuli such as temperature, pH, light, or electric field. Among these stimuli temperature and pH are the most important ones because of their physiological significance.[11,12]

Hydrogels have been studied as effective drug delivery vehicle for a long time and attracted increasing interests since the introducing of stimuli responsive properties.[13–15] Incorporation of pH and temperature responses into one network endows the hydrogels with dually responsive properties, which provides versatile ways to control the drug release. Based on this concept, a variety of hydrogels being sensitive to both pH and temperature have been synthesized.[16–19] Although there are many polymers responding to temperature change, poly(*N*-isopropylacrylamide) (PNIPAM) has been the most popular one used as the thermo-sensitive part due to its sharp phase transition.[20] PNIPAM chain undergoes a reversible coil-to-globule transition at about 32°C (lower critical solution temperature, LCST), below which it is soluble in water and above which it precipitates out from the solution. Hydrogels made of PNIPAM exhibited unique hydrophilic/hydrophobic changes around the LCST of PNIPAM leading to the reversible swelling and shrinking behaviors, which can be used as effective trigger to modulate drug release.[21–24] However, the most serious disadvantage is that PNIPAM hydrogels are not biodegradable. There have been many successful efforts to prepare PNIPAM-based biodegradable hydrogels by using

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biodegradable crosslinkers containing dextran or polylactide (PLA) units.[17,25,26] To the best of our knowledge, there have been no pH-sensitive polypeptide-based crosslinkers employed to crosslink PNIPAM up to now.

In view of these aspects, a promising strategy for designing biodegradable thermo- and pH-sensitive hydrogels was developed. In this work, PNIPAM was crosslinked by poly(L-glutamic acid) (PGA) modified with pendant double bond to endow hydrogel with both thermo- and pH-sensitive properties. PGA and its derivatives are most widely studied pH-sensitive polypeptides due to their biocompatibility and biodegradability.[27–31] In our previous work, a series of thermo- and pH-sensitive hydrogels composed of PGA and poly(NIPAM-*co*-HEMA) (PNH) were synthesized through chemical coupling between the carboxylic groups in PGA and the hydroxyl groups in PNH.[32] Although the hydrogels exhibited phase transition around the LCST reflected by optical transmittance change, their swelling ratios were not sensitive to temperature in neutral or basic media. To obtain hydrogels with improved thermosensitive properties, we design a new synthetic strategy, that is, copolymerized NIPAM with PGA macromonomer containing multiple 2-hydroxyethyl methacrylate (HEMA) units at different feeding weight ratios using a photoinitiation technique. The hydrogels possessed similar structure with PGA/PNH hydrogels. However, their temperature-sensitive properties were quite different from PGA/PNH hydrogels due to the different crosslinking mechanism. The as-prepared hydrogels shrank at temperature above their collapse temperature (CT) and reswelled when temperature was lowered to 25°C. And their pH-sensitive swelling behaviors were also examined at different temperature. Bovine serum albumin (BSA) were used as a model protein drug and loaded into the hydrogels. The pH and temperature depending release behaviors indicated promising application of these materials as controlled drug delivery vehicles.

EXPERIMENTAL SECTION

Materials

N-Isopropylacrylamide (NIPAM, 99%, Sigma, St. Louis, MO, USA) was recrystallized from hexane and dried under vacuum for 24 h prior to use. γ-Benzyl-L-glutamate *N*-carboxyanhydride (BLG-NCA) was prepared according to our previous method.[33,34] 33 wt % HBr solution in acetic acid (Acros), 4-dimethylaminopyridine (DMA, 98%, Fluka, Ronkonkoma, NY, USA) *N*,*N*′-dicyclohexyl carbodiimide (DCC, Sigma), and dichloroacetic acid (Shanghai Laize Chemical Co., China) were used as received. The cytocompatible UV photoinitiator Irgacure 2959 (I2959, 2-hydroxy-1-[4-(hydroxyethoxy) phenyl]-2-methyl-1-propanone) was obtained from Ciba-Geigy Chemical Co. (Tom River, NJ). HEMA (96%, Acros) was distilled under reduced pressure before use. Dimethyl sulfoxide (DMSO) was purified by vacuum distillation over CaH_2. Triethylamine was dried over CaH_2 and distilled prior to use. The simulated gastric fluid (SGF) was prepared as described in the United States Pharmacopoeia consisted of 3.2 mg/mL pepsin in 0.03*M* NaCl at pH 1.2. The simulated intestinal fluid (SIF) was also prepared as described in the United States Pharmacopoeia consisted of 10 mg/mL of pancreatin in 0.05*M* KH_2PO_4 at pH 6.8.

Synthesis of poly(L-glutamic acid)

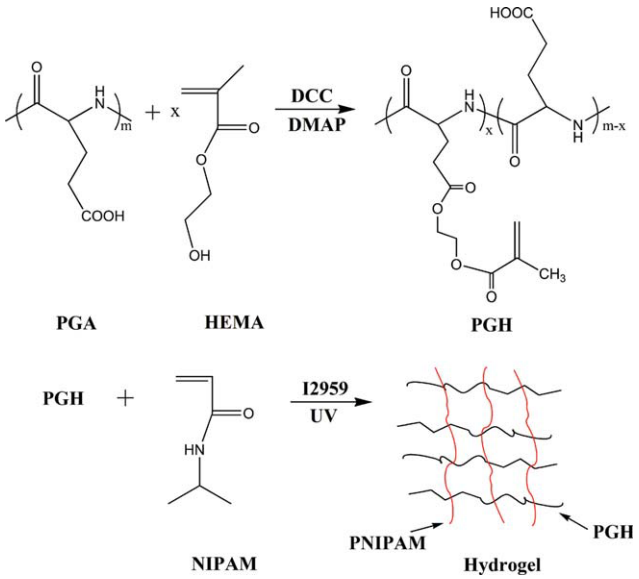
PGA was prepared according to our previous method.[34] Firstly, Poly(γ-benzyl-L-glutamate) (PBLG) was prepared by the ring-opening polymerization of BLG-NCA initiated by triethylamine with a monomer/initiator ratio of 25/1 at room temperature. After 3 days, the reaction mixture was added to a 10-fold excess of ethanol. The precipitated polypeptide was isolated by filtration and dried under vacuum at 40°C for 24 h.

PGA was prepared by debenzylation of PBLG using HBr. Briefly, PBLG (5 g) was dissolved in dichloroacetic acid (50 mL) at 30°C. After 25 mL 33 wt % HBr solution in acetic acid was added, the solution was stirred at 30°C for 1 h. Then the product was precipitated by pouring solution into excessive acetone, isolated by filtration, and repeatedly washed using acetone. Then the product was dried under vacuum at room temperature for 24 h.

δ (CF_3COOD, ppm): 4.6 (1H, -COC***H***NH-), 1.93 and 2.11 (2H, -C***H***$_2$$CH_2COOH$), 2.42 (2H, -$CH_2$C***H***$_2$COOH). The intrinsic viscosity and molecular weight of PGA were measured by using Ubbelohde type viscometer. The molecular weight of PGA determined from the intrinsic viscosity was 1.2×10^5.

Synthesis of poly(L-glutamic acid)-*g*-2-hydroxyethyl methacrylate

poly(L-glutamic acid)-*g*-2-hydroxyethyl methacrylate (PGH) was prepared by coupling reaction between the pendant carboxylic groups of PGA and hydroxyl groups of HEMA, as shown in Scheme 1. Typically, PGA (1.0 g) and HEMA (0.1 mL) were dissolved in dry DMSO (40 mL) in a flask at room temperature. The esterification of PGA with HEMA was started by adding 5 mL of a solution of DCC (0.2 g, at a molar ratio of 1.2 : 1 to HEMA) and DMAP (10 mg, at a molar ratio of 1 : 10 to HEMA) in DMSO to the flask. After 24 h, the mixture was filtered to remove precipitated dicyclohexylurea (DCU). Then the



Scheme 1 Synthetic routes of PGH and hydrogel. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

solution was poured into excess chloroform to precipitate PGH. The product was isolated by filtration, repeatedly washed by chloroform, and dried under vacuum at 35°C for 48 h.

Preparation of hydrogels

Hydrogels were prepared at room temperature using I2959 as the photoinitiator. Briefly, NIPAM and PGH (a total amount of 100 mg) at different weight ratios, (PGH : NIPAM) of 3 : 7, 4 : 6, 5 : 5, and 6 : 4 (w/w), were dissolved in 0.9 mL of 0.05*M* phosphate buffer (pH 7.4). After adding 0.1 mL of a solution containing of 1 mg I2959, the solution was placed into a cylindrical tube with a diameter of 8 mm and exposed to irradiation (5 mW/cm^2) at 365 nm provided by a UV light supplier. The reaction was allowed to proceed for 5 min to form hydrogel. The gel was then cut into a piece with a thickness of 8 mm, and the pieces were immersed in deionized water which was changed twice one day for 2 days. Then the swelled gel was lyophilized to obtain a dry gel. Hydrogels were denoted as Gel 3/7, 4/6, 5/5, and 6/4 corresponding to their initial PGH : NIPAM feeding ratios.

Characterization

^{1}H NMR spectra were recorded by a Bruker 300 MHz spectrometer. FTIR spectra were measured on a Bruker Vertex 70 Fourier Transform Infrared spectrometer using the KBr disk method. The morphology of the hydrogel was investigated by environmental scanning electron microscopy (ESEM) on an XL 30 ESEM FEG Scanning Electron Microscope (Micrion FEI PHILIPS). The hydrogel samples were swollen in deionized water at 25°C for 48 h to reach equilibrium, and then quickly put into liquid nitrogen for 10 min and transferred to a freeze-dryer for 72 h. The samples were then loaded on the surface of a copper SEM specimen holder and sputter coated with gold before observation.

The molecular weight of the PGA was estimated by viscosity measurement in 0.4*M* NaCl and 0.01*M* NaH_2PO_4 solution at pH 6.8 and 25.5°C using the intrinsic viscosity–molecular weight relationship derived from Hawkins and Holtzer[35]:

$$[\eta] = 2.93 \times 10^{-5} M^{0.923} \quad (1)$$

Swelling of hydrogels at different pH and temperatures

The dried sample was immersed in various solutions with certain pH and temperature for 2 days to reach equilibrium. The buffer solution was replaced frequently throughout the swelling process to ensure complete equilibration at the desired pH. The buffer solutions included the following: HCl solution (pH = 1.2, containing 0.03*M* NaCl), phosphate-buffered saline (pH 7.4), and 0.01*M* boric acid-buffered saline (pH 9.0). The ionic strength was adjusted with NaCl for different condition. The swelling ratio SR of hydrogels was calculated from the following equation:

$$\mathrm{SR} = (W_t - W_0)/W_0 \quad (2)$$

where W_t and W_0 are the weights of swollen gels and dried samples, respectively. All experiments were carried out in triplicate, and the average values were reported.

In vitro enzymatic degradation of hydrogels

The enzymatic degradation of hydrogels was carried out in SGF and SIF. The test tube was incubated at 37°C under constant shaking (100 rpm). At different time intervals, the samples were taken out and rinsed thoroughly with deionized water; then they were lyophilized to determine dry weights of hydrogels. In order to maintain enzymatic activity, the SGF was replaced every 4 h and the SIF was changed once a day. The percentage of weight loss [W_l (%)] was calculated based on following equation:

$$W_l(\%) = (W_0 - W_d)/W_0 \times 100 \quad (3)$$

where W_0 is original weight of the dried gel sample before immersion, and W_d is weight of the dried sample after degradation at predetermined time.

TABLE I
Feed and Result Compositions of PGH and the Yield, Swelling Ratio of Resulted Hydrogels

Sample	Feed HEMA/GA (mol/mol)	Graft ratio[a]	Yield of hydrogel[b] (%)	Swelling ratio[c] 25°C	Swelling ratio[c] 37°C
PGH1	10.6/100	7.5 %	51.3	31.5	26.2
PGH2	16.0/100	14.6 %	63.2	25.7	12.8
PGH3	21.3/100	19.9 %	68.5	9.4	5.5

[a] Determined by ^{1}H NMR.
[b] Feed ratio of PGH was 50 wt %.
[c] At pH 7.4, $I = 0.15M$.

Drug loading and release

BSA was loaded into hydrogels during the synthesis. The drug concentration was 5 mg/mL. Then the cylindrical hydrogel was dried in air without washing. For drug release studies, the drug-loaded hydrogels were immersed in acidic solution with pH = 1.2 or in 0.05*M* KH_2PO_4 buffer at pH 6.8. The samples were incubated at 37°C or 25°C under constant shaking (100 rpm). At selected time intervals, 0.4 mL of release media was withdrawn and replaced with 0.4 mL of fresh solvent. The amount of released protein was quantified by UV/Vis spectrophotometer (Shimadzu UV-2401PC) at 280 nm.

RESULTS AND DISCUSSION

Synthesis and characterization

To functionalize PGA with double bond pendants, HEMA was chemically coupled to its side carboxyl using DCC and DMAP. The chemical structures of PGH and hydrogels are shown in Scheme 1. ^{1}H NMR spectrum presented in Figure S1 (Supporting information) confirmed the successful synthesis of PGH. The mole content of HEMA in PGH was calculated from integration ratio between the characteristic peak of PGA at δ = 4.2 (–NHC***H***CO–) and the typical peak of HEMA appearing at δ = 5.4 ppm (***H***—CH=CCH_3–). In our synthetic strategy, HEMA content in PGH is directly associated with the crosslinking density of the resulting hydrogels. Therefore, we prepared a series of PGH containing different molar percentage of HEMA to evaluate the effect of HEMA content on hydrogels swelling behaviors.

As shown in Table I, the graft reaction was very efficient. However, the grafting of HEMA could not be achieved efficiently without DMAP in our preliminary experiments, indicating that DMAP played an important role in this condensation reaction due to its high catalysis efficiency.

Hydrogels were prepared by photoinitiated crosslinking reaction (Scheme 1). In this process, PGH acted as a crosslinker. On the other hand, PGH also provided hydrogels with pH-sensitive property. UV irradiation is a convenient and effective method to prepare cross-linked networks for biomedical purposes. It can provide several advantages such as controllable reactions kinetics in space and time, ability to uniformly encapsulate cells and drugs, and low cost. I2959 was chosen as an initiator because it was proved to be well tolerated by many cell types and will cause little damage to human body.[36] The structure of hydrogel was confirmed by FTIR spectra as shown in Figure 1.

The disappearance of peak at 940 cm^{-1} (wagging –C=C—H of PGH) verified the radical polymerization of its double bonds, which led to the formation of inter- and intra-polymeric cross-linking network. In the spectrum of hydrogel, the appearance of symmetric C—H bending from –$CH(CH_3)_2$ group (1368 and 1388 cm^{-1}) of PNIPAM and the strengthening of amide peak (1650 cm^{-1} and 1545 cm^{-1}) confirmed that PNIPAM was also successfully incorporated into network. Hydrogels consisted of 50 wt % PGH with different HEMA contents were synthesized. And the effect of HEMA contents on swelling ratio of hydrogels was investigated and the results were

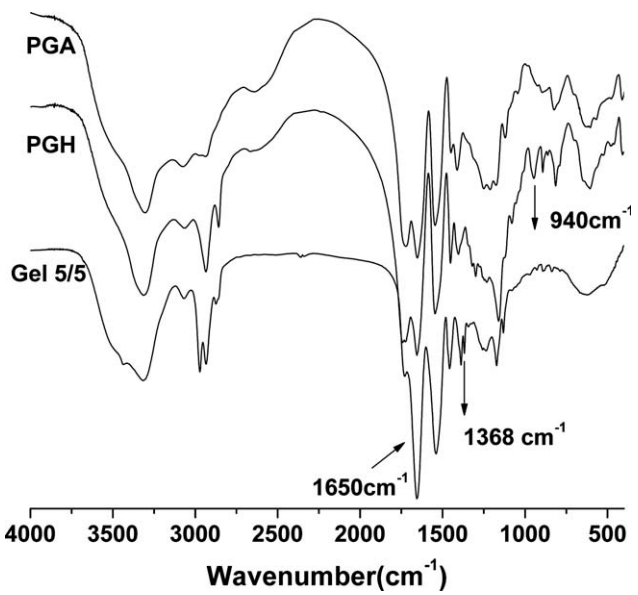


Figure 1 FTIR spectra of PGA, PGH, and Gel 5/5.

TABLE II
Feed Compositions, Yields, and Diffusional Exponent (*n*) of Hydrogels

Sample	PGH2 (mg)	NIPAM (mg)	I2959 (mg)	Yield (%)	*n* 25°C	*n* 37°C
Gel 3/7	30	70	1	61.0	0.46	0.55
Gel 4/6	40	60	1	61.8	0.49	0.76
Gel 5/5	50	50	1	63.2	0.50	0.59
Gel 6/4	60	40	1	64.1	0.56	0.77

shown in Table I. It was found that a higher HEMA content led to a lower swelling ratio, which was caused by a higher crosslinking density. Moreover, hydrogels prepared by PGH1 had weak thermo-sensitive shrinking. Therefore, PGH2 was chosen for further preparation of hydrogels containing different PGH percentages. As listed in Table II, the yields of hydrogels increased with the increase of PGH content. This could be attributed to the increasing crosslinking density of hydrogels associated with PGH content.

Swelling behaviors

The swelling kinetic behaviors of hydrogels at different temperatures were investigated and shown in Figure 2. At 25°C, all hydrogels exhibited rapid initial swelling profiles in the first several hours due to highly hydrophilic nature of the hydrogels. The maximum swelling ratios of hydrogels at 25°C and pH 7.4 were in the sequence of Gel 4/6 > Gel 6/4 > Gel 3/7 ≈ Gel 5/5. PGA is a polyelectrolyte with carboxyl pendant which could dissociate to $-COO^-$ in an alkaline media. Thus, hydrogels containing more PGH will have a higher swelling ratio at pH 7.4 due to the strong electrostatic repulsions among carboxylate anions. However, Gel 4/6 had a higher swelling ratio despite its lower PGH content than those of Gel 6/4 and Gel 5/5. It is well known that a higher cross-linking density would result in a lower swelling ratio.[37] In this work, hydrogels containing more PGH had higher cross-linking density. In addition, the content of PNIPAM also should be taken into account, which can be reflected by the order of hydrogels' swelling ratios (*vide infra*). Therefore, it can be inferred that the highest swelling ratio of Gel 4/6 at 25°C should be attributed to the combined effect of PNIPAM content, crosslinking density, and electrostatic repulsions. When temperature increased to 37°C, hydrogels reached to their maximum swelling more rapidly and the maximum swelling ratios increased in the order of Gel 3/7 < Gel 4/6 < Gel 5/5 < Gel 6/4. This result was in accordance with PNIPAM content in hydrogels which determined the thermo-sensitive extent of hydrogels. The water absorption curve as a function of time can be simulated using following equation (valid in the range of $M_t / M_\infty < 0.6$):

$$M_t/M_\infty = kt^n \tag{4}$$

where M_t is the mass of water absorbed by hydrogel at time t, M_∞ is mass of water absorbed at equilibrium, k is a characteristic constant of hydrogel, and n is the diffusional exponent.[38–40] The value of n can indicate diffusion mechanism of a particular system. For cylindrical hydrogels, the values of n have following conceptual meanings: (i) $n = 0.45$ for Fickian diffusion (Case I), (ii) $0.45 < n < 0.89$ for anomalous transport (combined contribution of Fickian diffusion and controlled-relaxation), and (iii) $n = 0.89$ for zero order (Case II, controlled-relaxation of the polymer chains). Table II shows that n values of all hydrogels at both 25°C and 37°C are in a range of $0.45 < n < 0.89$ indicating an anomalous transport mechanism. It should be noted that the values of n at 37°C are

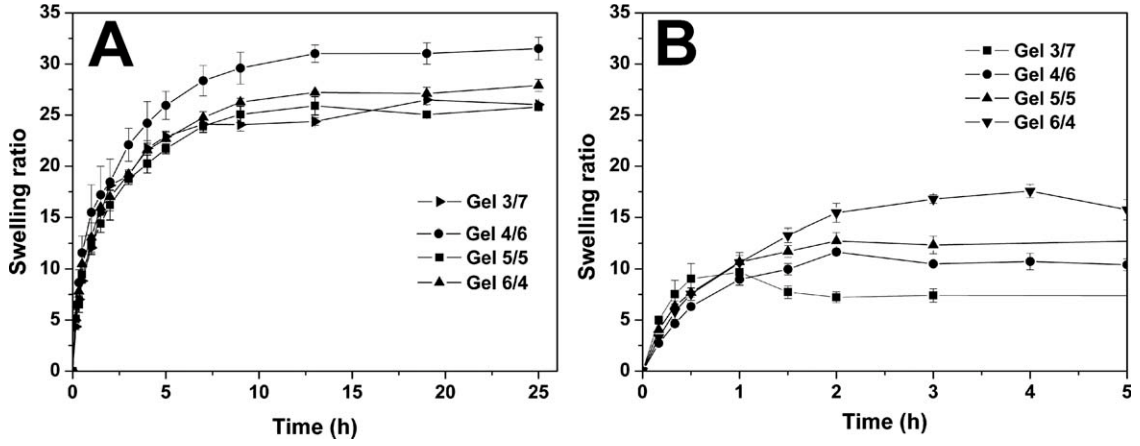


Figure 2 Swelling kinetics of hydrogels at 25°C (A) and 37°C (B), pH 7.4, $I = 0.15M$. All the results are based on $n = 3$ measurement.

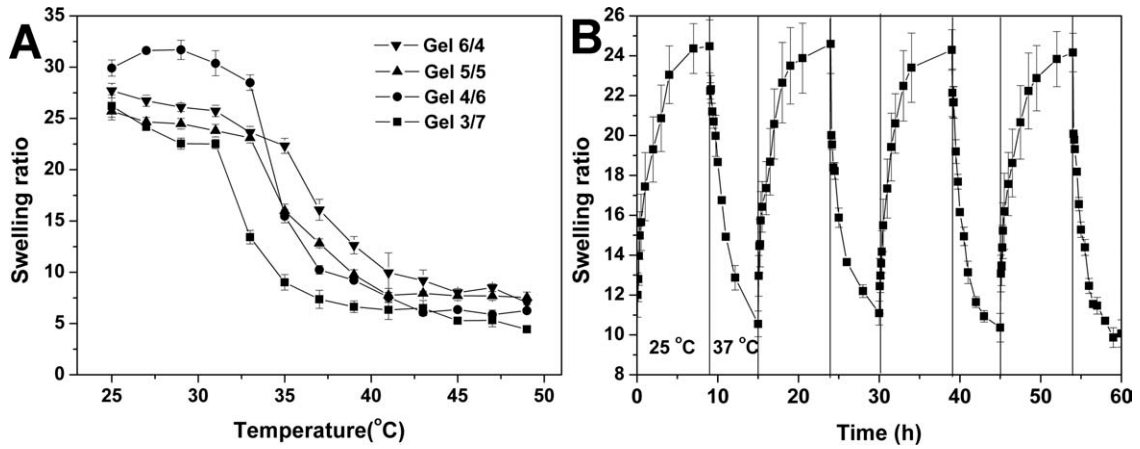


Figure 3 Thermo-sensitive behaviors of hydrogels at different temperatures (A) and reversible swelling–deswelling behaviors of Gel 5/5 at 25°C and 37°C, pH 7.4, $I = 0.15M$ (B, the samples are immediately immersed into buffer at 37°C after taking out from buffer at 25°C, or otherwise). All the results are based on $n = 3$ measurement.

higher than that at 25°C, which indicates that swelling process became more dependent on the polymer relaxation at a higher temperature. It could be understood that the increasing hydrophobic intra- and interaction of PNIPAM at a temperature above the LCST restrict relaxation of polymer network.

Figure 3A demonstrated temperature dependent swelling of hydrogels from 25°C to 49°C. As expected, all hydrogels exhibited reduced swelling ratios above the LCST of PNIPAM at around 32°C due to phase transition behavior of PNIPAM. In our previous work, hydrogels with similar structure had little swelling ratios difference between 37°C and 25°C.[32] Probably, it was caused by defective network structure of that kind of hydrogel. In that work, the crosslinking was achieved by condensation reaction which will become hard to continue as the reaction proceeding because increasing viscosity of the system made it difficult for diffusion of polymer chain. Thus, crosslinking density of those hydrogels is relatively low and phase transition of PNH will not result in constriction of the hydrogel network but only cause chain rearrangement. In this report, the crosslinking reaction was achieved by the photoinitiated polymerization which has higher efficiency. Therefore, PNIPAM chain has more crosslinking point with PGA chain which will be favorable to cause network shrinkage after phase transition of PNIPAM leading to the deswelling of hydrogels. Generally, the CT of hydrogels was defined as the temperature at which swelling ratio starts to decrease sharply. It can be seen in Figure 3A that the CT of hydrogels increased with the PGH content increase, which was attributed to the increasing hydrophilicity caused by PGH. This result indicated that it was convenient to adjust the CT of hydrogels using different feed ratios of NIPAM and PGH for specific application.

The swelling–deswelling kinetics of hydrogels at different temperatures was important for its potential application for pulsatile drug delivery and was investigated as shown in Figure 3B. The hydrogels

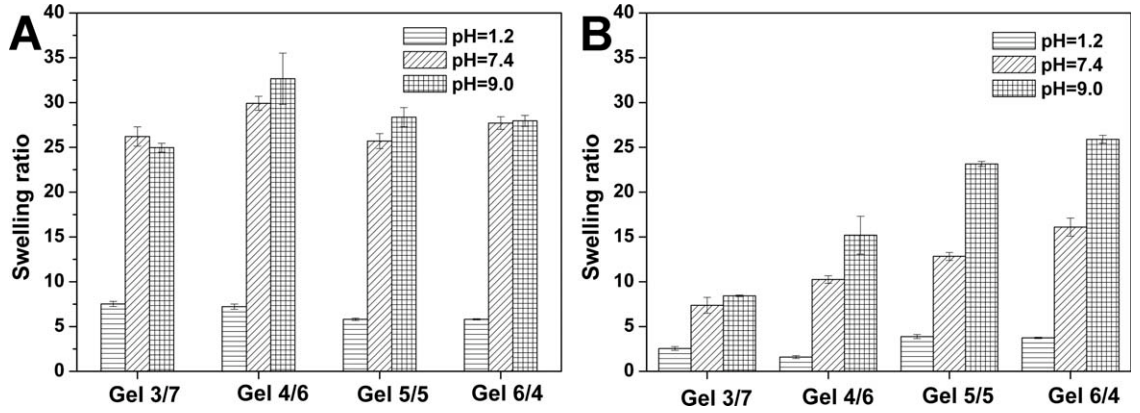


Figure 4 pH-responsive behaviors of hydrogels at pH 1.2 (containing 0.03*M* NaCl), pH 7.4 ($I = 0.15M$), pH 9.0 ($I = 0.15M$) and 25°C (A) and 37°C (B). All the results are based on $n = 3$ measurement.

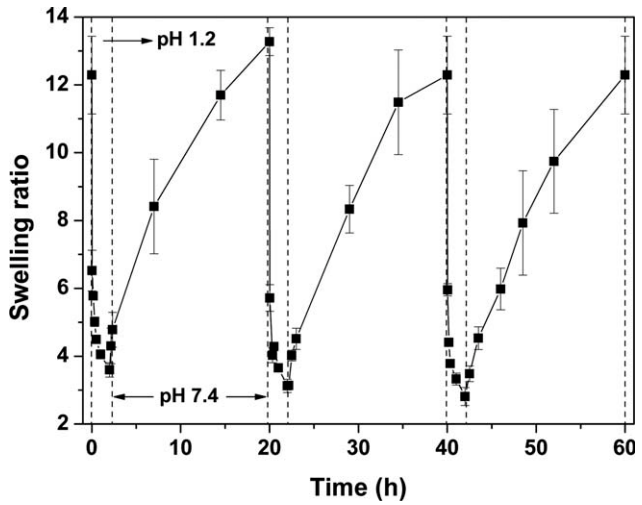


Figure 5 Reversible swelling–deswelling behaviors of Gel 5/5 at pH 1.2 (containing 0.03*M* NaCl) and pH 7.4 (I = 0.15*M*), 37°C. The samples are absorbed with filter paper to remove the surface buffer solution and immediately immersed into buffer at pH 1.2 after taking out from buffer at pH 7.4, or otherwise. All the results are based on $n = 3$ measurement.

showed excellent reversible swelling behaviors as temperature changes and the responsive rate did not change greatly after several cycles.

PGA is one of a most used polypeptide bearing pendant carboxyl due to its favorable biocompatibility and biodegradability. Therefore, incorporating PGH into the network endowed hydrogels with pH responsive ability. The swelling ratios of hydrogels at different pH and temperatures were shown in Figure 4. The hydrogels exhibited similar swelling behaviors at 25°C at different pH. For example, all hydrogels had higher swelling ratios at both pH 7.4 and 9.0 than those at pH 1.2. Moreover, there were no significant differences in swelling ratios for all hydrogels between pH 7.4 and pH 9.0. However, when temperature was elevated to 37°C, the swelling ratios were very different. For hydrogels with given PGH content, swelling ratios increased when pH increased from pH 7.4 and pH 9.0. The different swelling behaviors at basic pH values for 25°C and 37°C can be attributed to phase transition of PNIPAM. At 37°C PNIPAM turned to be hydrophobic and the swelling ratios of hydrogels were mainly controlled by pH values, so swelling ratios increased as pH increased from 7.4 to 9.0. While at 25°C PNIPAM exhibited hydrophilic properties and would dilute the effect of pH on swelling ratios, thus the swelling ratio differences between pH 7.4 and 9.0 were not great.

The pH-responsive swelling–deswelling property of hydrogels was demonstrated by Gel 5/5 between pH 1.2 and 7.4 at 37°C. As shown in Figure 5, the swelling–deswelling process was repeatable as pH change across cycles and deswelling rate was faster than re-swelling rate, indicating the compact structure formed after deswelling restricted deprotonation and re-extension of network.

It was well known that hydrogel containing polyelectrolyte exhibited ionic strength responsive property.[13] In this work, the hydrogels were also sensitive to temperature. Therefore, it was necessary to investigate swelling behaviors of hydrogels at different ionic strengths and temperatures. The results were shown in Figure 6. As expected, all hydrogels shrank as ionic strength increase at both 25°C and 37°C. But the sensitivity was weakened when temperature increased from 25°C to 37°C. That could be attributed to relatively low swelling ratio at temperature above CT diluted the shrinking extent.

Hydrogel morphology

To examine the interior structure of hydrogel in a swollen state, ESEM measurement was performed. SEM technique has been employed to reveal hydrogel structure for a long time. It should be noticed

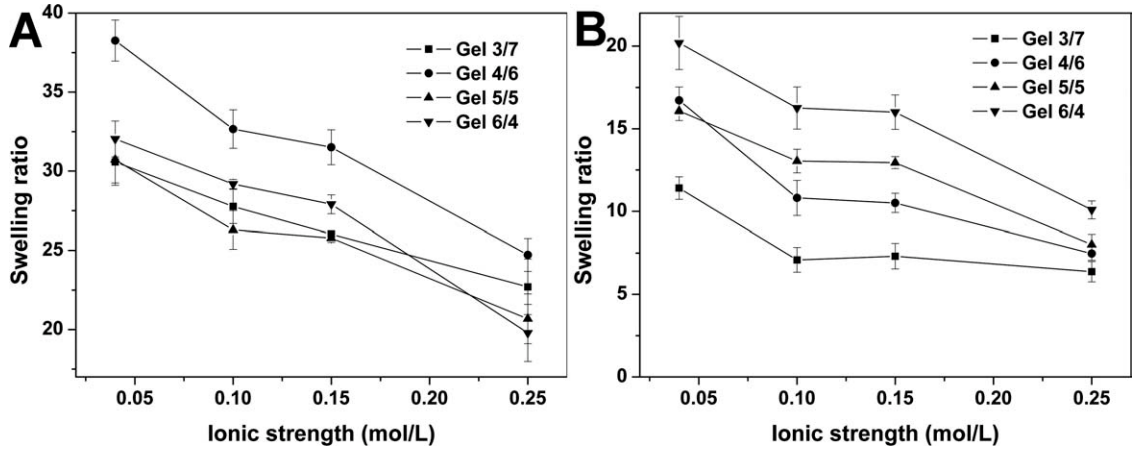


Figure 6 Ionic strength-sensitive behaviors of hydrogels at pH 7.4, 25°C (A) and 37°C (B). All the results are based on n = 3 measurement.

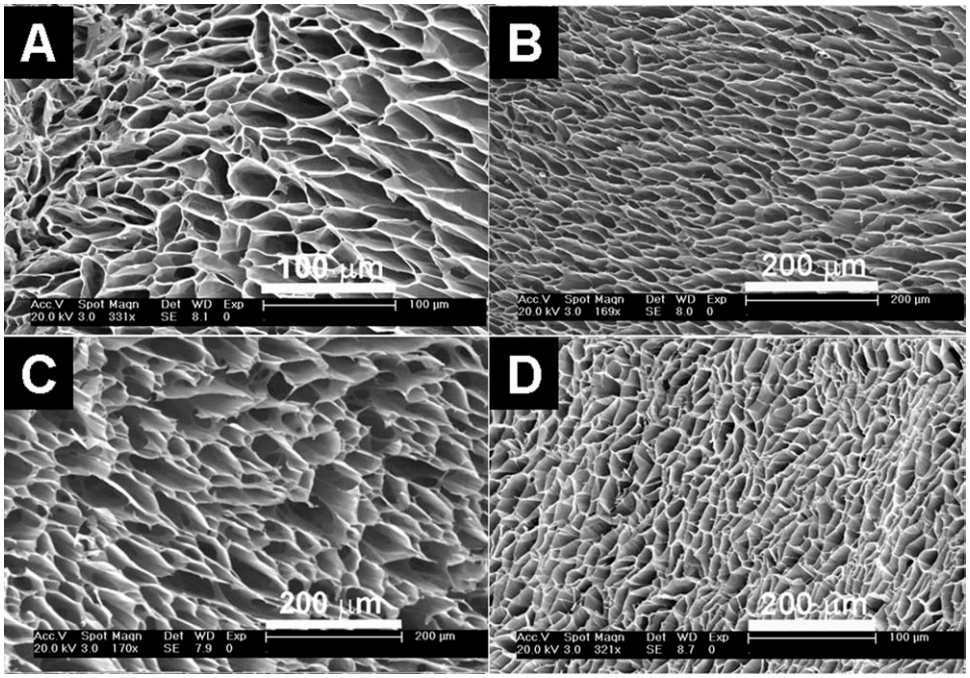


Figure 7 SEM micrographs of Gel 6/4 (A), Gel 5/5 (B), Gel 4/6 (C), and Gel 3/7 (D).

that the pretreatment procedures for SEM examination also affect morphology of a hydrogel.[41] As shown in Figure 7, all of hydrogels exhibited regular and porous structures, which clearly indicated that hydrogels were homogeneous and no obvious phase separation occurred in the network. The pore sizes of hydrogels were in the order of Gel 4/6 > Gel 6/4 > Gel 3/7 > Gel 5/5, which was in accordance with their swelling ratios.

In vitro biodegradability of hydrogels

PGA is one of the most studied synthetic polypeptide and has been proved to be biodegradable *in vitro* and *in vivo*. We investigated the degradability of hydrogels composed of PGA in the SGF and SIF medium *in vitro*. As a hydrogel had potential application in the oral drug delivery system, it should maintain its network structure in the gastric condition and be degraded in the intestinal condition. Therefore, the degradation behaviors of hydrogels in SGF and SIF were studied and the results were shown in Figure 8. In our experiments, all hydrogels exhibited no weight loss in SGF after 28 h, which avoided the burst release of drug caused by the hydrogel degradation in the stomach. When the hydrogels were transferred into the SIF, the degradation took place rapidly as the hydrogels swelling and finally the hydrogels were broken down. This degradation behavior had promising

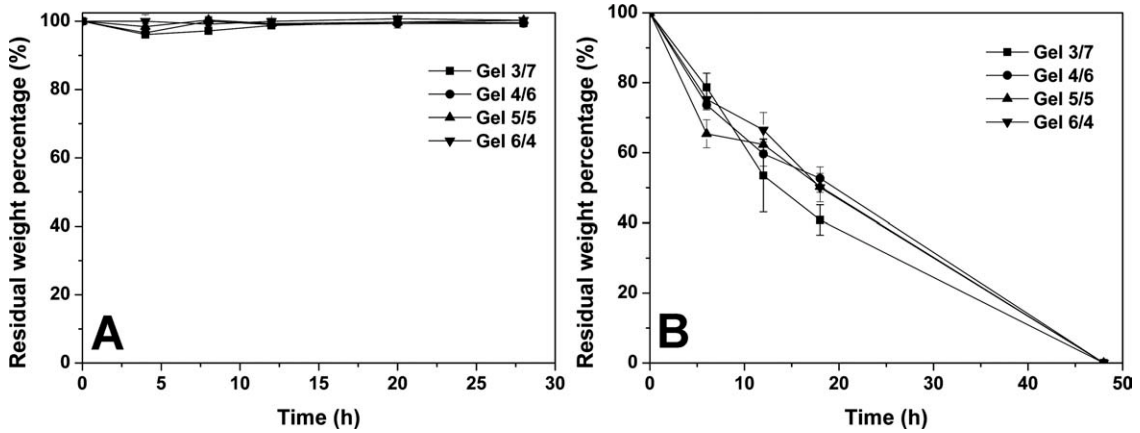


Figure 8 Degradation behaviors of hydrogels in SGF (A) and SIF (B). All the results are based on $n = 3$ measurement.

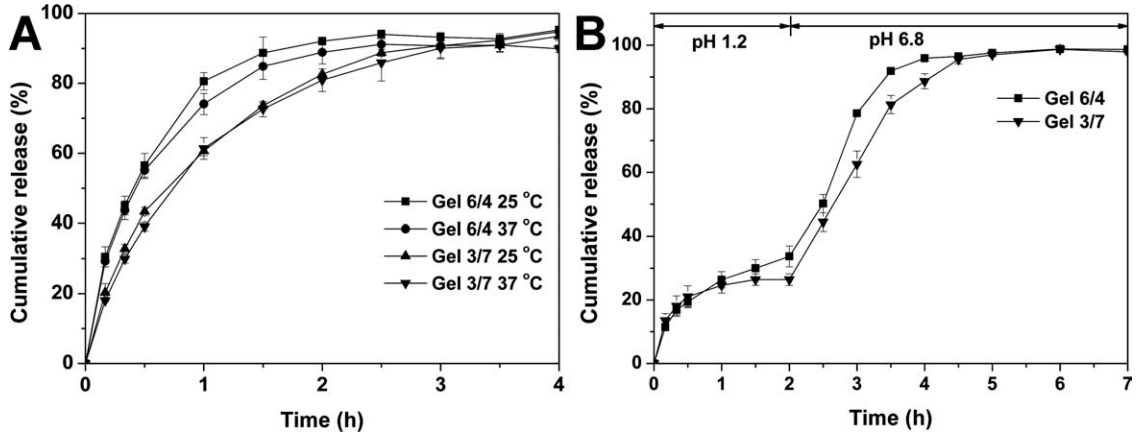


Figure 9 BSA release profiles of Gel 6/4 and Gel 3/7 in pH = 6.8 buffer at 25°C and 37°C (A), and BSA release profiles of Gel 6/4 and Gel 3/7 in pH = 1.2 solution for 2 h and subsequently in pH = 6.8 buffer for additional 5 h at 37°C (B). All the results are based on $n = 3$ measurement.

application in the oral delivery of protein drug because it allowed the protection of drugs in the stomach and rapid release of drugs in the intestine.

In vitro release of BSA

In vitro BSA release profiles of hydrogels were investigated at different temperature and pH. As shown in Figure 9A, both Gel 6/4 and Gel 3/7 exhibited gradual release of BSA and model drug was almost completely released after 4 h. During the initial 2.5 hours, Gel 6/4 had faster release rate with a cumulative release of 94% and Gel 3/7 was the second with a cumulative release of 88%. The isoelectric point (pI) of BSA was around 4.7 and at pH 6.8 it carried net negative charge. At pH 6.8 PGA also carried negative charge due to ionization of its pendent carboxyl. Therefore, repulsion force between BSA and network also plays important role in release process. Figure 9A also exhibited cumulative release of BSA from hydrogels at temperature above LCST of PNIPAM. The release profiles at 37°C were similar as those at 25°C while release rates were slower than those of 25°C. And the amounts of the cumulative release at the end of investigated period were also lower than those at 25°C. The hydrophobic interaction of PNIPAM in hydrogels at 37°C might hinder diffusion of BSA and hence hydrogels containing more PNIPAM exhibited a slower BSA release rate.

PGA is most widely studied polypeptides for its pH-sensitive property, so it is necessary to examine BSA release profile under an acidic condition. As shown in Figure 9B, the release rates of both hydrogel were slower at pH 1.2 at initial 2 h than at pH 6.8 media. For example, Gel 3/7 had 76% BSA released after 2 h at pH 6.8 and only 22% was released at the corresponding time at pH 1.2. PGA is protonated under an acidic condition which will restrict diffusion of drugs due to its constrained network. The release rate of Gel 3/7 was slower than Gel 6/4 at pH 1.2, which may be attributed to combined effect of PGA and PNIPAM because both of them are hydrophobic at pH 1.2 and 37°C. After the hydrogels were transferred into pH 6.8 media, they began to swell and pore size increased leading to rapidly release of BSA.

CONCLUSION

A series of pH- and thermo-sensitive hydrogels were synthesized by crosslinking of PNIPAM using a biodegradable pH-sensitive crosslinker. The swelling behaviors of hydrogels were investigated at different pH and temperatures. The hydrogels shrank under an acidic condition or at a temperature above the CT and would swell at neutral or basic media or at a lower temperature. The swelling/deswelling behaviors were reversible. ESEM observations revealed that all hydrogels had similar porous structures. In the SGF condition, all hydrogels maintained their network structures and were degraded rapidly in the intestinal condition. The *in vitro* release of BSA from hydrogels at pH 6.8 and different temperature demonstrated that hydrogels had a slower release rate at temperature above their CT. For different pH media, the release rates of hydrogels were slow at pH 1.2 and increased sharply after they were transferred into pH 6.8 buffer. The results indicated promising application of these materials as stimuli-responsive drug delivery vehicles.

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