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ORIGINAL CONTRIBUTION

Preparation and release profiles of pH/temperature-responsive carboxymethyl chitosan/P(2-(dimethylamino) ethyl methacrylate) semi-IPN amphoteric hydrogel

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Abstract Thermo- and pH-responsive semi-IPN polyampholyte hydrogels were prepared by using carboxymethyl chitosan and P(2-(dimethylamino) ethyl methacrylate) with *NN*'-Methylenebisacrylamide (BIS) as crosslinking agent. It was found that the semi-IPN hydrogel shrunk most at the isoelectric point (IEP) and swelled when pH deviated from the IEP. Its swelling ratio dramatically decreased between 30 and 50 °C at pH 6.8 buffer solution. It also showed good reversibility. The UV results showed that when the pH values of drug release medium were 3.7, 6.8, and 9 at 25 °C, the cumulative release rates reached 83.1, 51.5, and 72.2%, respectively. The release rate of coenzyme A (CoA) was higher at 50 °C than 37 and 25 °C at pH 6.8 solution. The release rate decreased with increasing the content of carboxymethyl chitosan at 25 °C in pH 6.8 solution. The results showed that semi-IPN hydrogel seems to be of great promise in pH/temperature drug delivery systems.

Keywords Biomaterials · Drug delivery systems · pH/temperature-responsive polymer · Semi-IPN polyampholyte hydrogel · Swelling behavior

Introduction

Amphoteric polyelectrolyte hydrogels possessing both positive and negative charges are therefore interesting synthetic analogs for proteins. It is known that undergoing continuous or discontinuous volume phase transition responses to solvent composition [1], pH [2], salt concentration [3], temperature [4], and ultraviolet light [5] has received much attention recently because of their scientific and technology importance [6–8]. Now, many researchers are using amphoteric polyelectrolyte hydrogels to develop artificial muscles for robots and human prosthesis, controlled delivery systems such as an insulin pump for diabetics, chemical valves that control the flow of liquids, sensors, actuators, matrices for molecular recognition or separation, etc [9–13]. Growing attention is currently being devoted to the stimuli-sensitive polymer hydrogels. Among these systems, pH- or temperature-responsive hydrogels have been extensively studied in the biomedical field because these two factors can be easily controlled and are applicable both in vitro and in vivo conditions [14–17].

Carboxymethyl chitosan (CM–CS), a natural amphoteric polyelectrolyte derived from chitosan has already been extensively used in a wide range of biomedical applications, such as wound dressings, artificial bone and skin, bacteriostatic agent and blood anticoagulants, due to its unique chemical, physical, and biological properties, especially its excellent biocompatibility [18–20]. It has also demonstrated good pH and ion sensitivity in aqueous solutions due to abundant –COOH and $–NH_2$ groups [21].

P(2-(dimethylamino)ethyl methacrylate)(PDMAEMA) with thermo and pH sensitivity at the same time [22, 23] is a widely used polymer in biomedical materials, such as drug delivery system, artificial skin, contact lens, and so on [24].

To combine the advantage of synthetic and natural polymers and at the same time maintain the favorite property of natural polymers such as biodegradation and bioactivity, amphoteric polyelectrolyte hydrogels with pH and temperature sensitivity were synthesized with CM–CS and PDMAEMA in this work. The swelling behavior of the semi-IPN hydrogel under different pH and temperature was

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studied. The release behavior of coenzyme A was also investigated.

Experimental

Materials

Chitosan (CS) was purchased from Tokyo Kasei Kogyo. The degree of deacetylation was 0.85 as measured by elemental analysis [25]. Carboxymethyl chitosan with 0.77 degree of substitution as determined by potentiometric titration [26] was prepared according to the literature method [27]. 2-(Dimethylamino)ethyl methacrylate (DMAEMA) and coenzyme A (CoA) were all purchased from Aldrich. *NN*'-Methylenebisacrylamide (BIS) as cross-linking agent, was purchased from Shanghai Reagent. Ammonium persulfate (APS) was obtained from Peking Chemical Industry, China, and was recrystallized before use. All other chemicals used were of analytical grade, without further purification.

Preparation of CM–CS/PDMAEMA semi-IPN hydrogels

Various ratios of DMAEMA, CM–CS, and 3 wt% BIS based on the total monomers were dissolved in 8 ml deionized water as described in Table 1. After bubbling N_2 gas for 30 min to deoxygenate of the solution, 1 wt% APS as redox initiators were added to the solution. Then, the mixture was incubated at 50 °C for 24 h. After the gelation was completed, the semi-IPN hydrogel was cut into disks and immersed in an excess amount of deionized water for 4 days to remove the residual unreacted monomers. Swollen semi-IPN hydrogels were dried in a vacuum oven for 3 days at 30 °C to a constant weight. The thickness of the dried hydrogel was about 1–1.5 mm and the diameter was about 4–5 mm.

Drug-loaded semi-IPN hydrogel was prepared using a similar method for release experiments, in which CoA was mixed with the solution at the ratio of 10% (*w*/*w*) (relatively to the total weights of CM–CS and DMAEMA), and then stirred gently for 1 h at room temperature before APS was added into the mixed solution.

Table 1 Feed composition for the preparation of semi-IPN hydrogels

Component	Sample code			
	PDMAEMA	Semi-IPN05	Semi-IPN10	Semi-IPN20
DMAEMA (g)	0.86	0.86	0.86	0.86
W (CM–CS) %)[a]	0	5	10	20
m (BIS) (g)	0.0258	0.0258	0.0258	0.0258
m (APS) (g)	0.0086	0.0086	0.0086	0.0086
V (H_2O) (ml)	8	8	8	8

[a] The concentration is based on the mass of monomer DMAEMA

Characterization

IR spectra of the hydrogel were recorded using KBr pellets on AVATAR-360FT-IR at a resolution of 4 cm^{-1}. The isoelectric point (IEP) of the mixture was estimated from potentiometric titration using a Delta-320-S pH meter (China). All the UV spectrum of the release medium was recorded with a UV–visible spectrophotometer (UV-540, US).

Swelling studies

The swelling ratio (SR) was determined by immersing the dry semi-IPN hydrogels in aqueous solutions of the desired pH or temperature in sealed containers. After regular periods of time, they were removed from the aqueous solution, after the removal of excess surface water with filter paper, weighed and returned to the same container until equilibrium was observed. SR was calculated from the equation: $SR = (W_s - W_d)/W_d$, where W_s and W_d represent the weights of the swollen and dry-state samples, respectively. The pH of the external solution was adjusted according to literature [28].

Swelling–deswelling–reswelling measurements

Swelling–deswelling–reswelling cycles of semi-IPN hydrogels were carried out at pH 3.7–9 or at temperatures around the volume phase transition temperature (VPTT) of the hydrogels. The hydrogels were immersed in pH 3.7 solutions for 2 h, afterwards in pH 6.8 solutions for 2 h, and immersed in pH 3.7 solutions for 2 h again. This cycle was continuously run. The weights of swollen hydrogels were recorded before each immersion. The SR values of cycle-swollen hydrogels were obtained through the above equation, and averaged using at least two samples of each hydrogel. Swelling–deswelling–reswelling measurements at different temperature was done as the same method.

Determination of drug-loading content and loading efficiency of the hydrogel

The semi-IPN hydrogel (10 mg) were kept in 100 ml water with pH 3.7 values at 25 °C under stirring for 72 h. After centrifugation, the amount of CoA was determined in the clear supernatant by UV spectrophotometer (UV-540, US) at 260 nm using a calibration curve constructed from a series of CoA solutions with standard concentrations. Such experiments allow the calculation of the drug-loading content (%) and the loading efficiency (%). The loading

content (%) of drug and the loading efficiency (%) were calculated as following equation:

$$\text{Drug loading content}(\%) = \frac{\text{amount of drug in the semi -IPN hydrogel}}{\text{mass of semi-IPN hydrogel}} \text{x}100$$

$$\text{Loading efficiency}(\%) = \frac{\text{amount drug loading}}{\text{theoretical loading}} \text{x}100$$

In vitro drug release

The release rate experiments were performed in a glass apparatus at 25, 37, and 50 °C under unstirred conditions, or in acidic (pH 3.7), neutral (pH 6.8), and alkaline (pH 9) solutions, respectively. At a time interval, 1 ml sample were withdrawn and assayed for the amount of released CoA as a function of time. The amount of released CoA was analyzed with a spectrophotometer as described previously. The experiments were done in triplicate.

Results and discussion

FTIR spectra of the semi-IPN hydrogel

The IR spectra of CS, CM–CS, PDMAEMA, and semi-IPN hydrogel CM–CS/PDMAEMA were shown as curves a, b, c, and d in Fig. 1. Curve a showed signals of nonmodified chitosan at 1,647 and 1,590 cm^{-1} for the C–O stretching (amide) and N–H bending (amine), respectively. The spectra of CM–CS (curve b) is similar to that of the original chitosan (curve a), while a new peak appeared at 1,718 cm^{-1} which is assigned to the carbonyl groups on the side chains. Compared to the IR spectra of semi-IPN hydrogel (d) and CM–CS (b), curve d has a new peak appearing around 1,728 cm^{-1} corresponding to the ester on the PDMAEMA. Compared to PDMAEMA (curve c), The IR spectra of semi-IPN hydrogel (d) also revealed a new peak at 3,400 cm^{-1}, which was assigned to –OH and NH_2 in the CM–CS. All the above characterization showed that we synthesized the semi-IPN hydrogel.

Swelling kinetics of different CM–CS content of semi-IPN hydrogel

Figure 2 shows the swelling kinetics of the various CM–CS/PDMAEMA semi-IPN hydrogels at 25 °C in pH 6.8 values solution. The swelling ratios of the hydrogel ranged from 16 to 48.4, and they changed in relation to the CM–

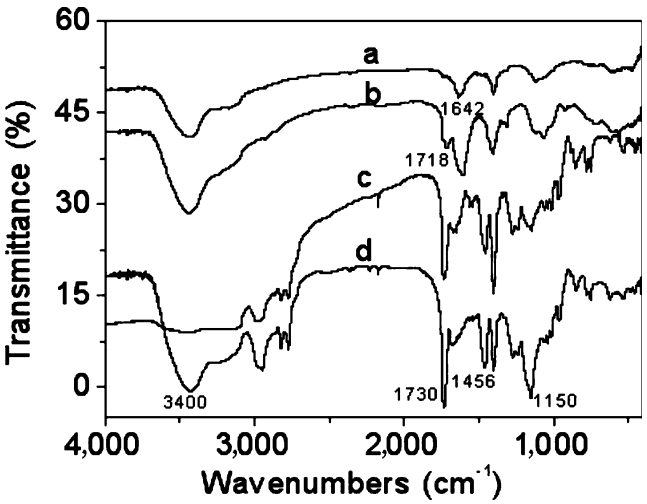

Fig. 1 FTIR spectrum of CS (*a*), CM–CS (*b*), PDMAEMA (*c*), and semi-IPN hydrogel (*d*)

CS and PDMAEMA network composition. The swelling ratio of PDMAEMA was higher than those of semi-IPN05, semi-IPN10, and semi-IPN20. An increase in the CM–CS fraction of the hydrogel was observed to lead to a decrease in the swelling ratio. This was attributed to the electrostatic attraction between the function groups. –COOH was partly changed into $–COO^-$, and $–NH_2$, $–N(CH_3)_2$ were partly positive charged in the polymer network at pH 6.8 solution. The electrostatic attraction between $–COO^-$ and $–NH_3^+$, $–NH^+(CH_3)_2$ make the hydrogel compact. In the experimental conditions, the content of PDMAEMA is a lot more than CM–CS. $–NH_3^+$ and $–NH^+(CH_3)_2$ were dominant in the hydrogel system. As a consequence, the electrostatic attraction between $–COO^-$ and $–NH_3^+$, $–NH^+(CH_3)_2$ increased with the fraction of CM–CS increasing at pH 6.8 solution. Moreover, it was also found that PDMAEMA

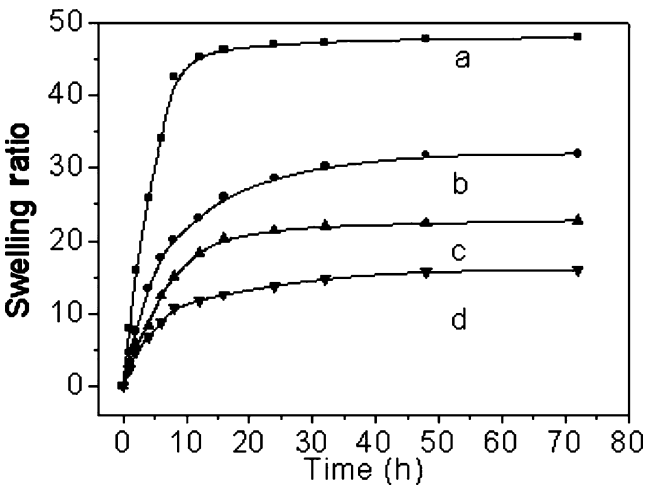

Fig. 2 Effect of CM–CS content on swelling ratio as a function of time at pH 6.8 values solution at 25 °C, *a* PDMAEMA; *b* semi-IPN05; *c* semi-IPN10; *d* semi-IPN20

samples swelled the most rapidly and reached equilibrium within 12 h. The time reached to equilibrium swelling became longer with the content of CM–CS increasing. This may be also related with the electrostatic attraction in the hydrogel system discussed above.

pH dependence of swelling

pH-sensitive hydrogels usually contain pendant acidic or basic groups, which change the ionization state in response to variations in pH. Semi-IPN hydrogel of CM–CS/PDMAEMA contains abundant $–COO^-$, $–NH_3^+$, and $–NH^+(CH_3)_2$ groups. To investigate the effect of pH, the sample semi-IPN10 was swollen in several buffer solutions with pH 1, 3.7, 5.5, 6.8, 9, 11, and 13. The ionic strength of the buffers was kept constant (I=0.5 M), since it will largely affect the swellability of the hydrogels. The IEP of the mixture of CM–CS and DMAEMA aqueous solutions was near pH 5.5 as estimated by potentiometric titration. The results in Fig. 3 showed that when the pH of the external solution deviated from the IEP, semi-IPN10 hydrogel behaved as polycations or polyanions, and SR increased. Near the IEP, the semi-IPN hydrogel shrunk to a minimum equilibrium volume showing a SR of 16. The swelling ratio dramatically increased when pH is lower than IEP, and SR slightly increased with the pH higher than IEP. These changes may be related to the electrostatic repulsion of CM–CS and PDMAEMA in the hydrogel network.

The driving force for the swelling and shrinking of polyelectrolyte hydrogel is the difference between the concentration of free ions inside and outside the hydrogel according to the Donnan equilibrium [29]. When the concentration of mobile ions inside the hydrogel is lower than in the surrounding condition, the osmotic pressure in the surrounding causes the hydrogel to shrink; otherwise, a larger osmotic pressure inside the hydrogel causes the hydrogel to swell. At the IEP, the numbers of $–NH_3^+$, $–NH^+(CH_3)_2$, and $–COO^-$ groups are equal, and intraionic attraction between opposite charges results in the lowest mobile ionic concentration in the hydrogel. When the pH deviates from the IEP, the amount of residual ionic concentration in the hydrogel increases gradually with increase or decrease of pH. However, in Fig. 3, the SR at pH 9 values solution is bigger than that in pH 13 values solution. A possible explanation may be the formation of new crosslinks by hydrogen bonding and hydrophobic interactions [30, 31]. At pH 13, the groups $–NH_3^+$ and $–NH^+(CH_3)_2$ were completely deprotonated and then contributed to the loss of solubility of the chain segments and to the hydrophobic interactions.

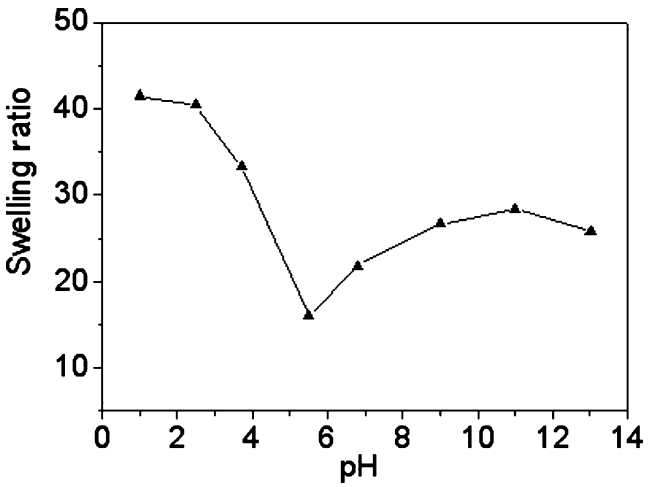

Fig. 3 Effect of pH on swelling ratio of hydrogel semi-IPN10

Because the semi-IPN hydrogel swell differently in different pH medium, we have investigated their pH-dependent swelling reversibility. The swelling reversibility of the semi-IPN10 at pH between 3.7 and 6.8 buffers, and pH between 6.8 and 9 buffers were shown in Fig. 4 curve a and b, respectively. Because the time interval between each step was 2 h, the experimental data in Fig. 4 are not equilibrium values. However, the data show that the largest changes in the swelling and deswelling behavior already occurred in the 2 h. It demonstrates the semi-IPN hydrogel reversibility to absorb and deabsorb water upon changing the pH in acidic and neutral region or in neutral and alkaline region. The results showed that the swelling–deswelling behavior of the semi- IPN hydrogel showed a good reversibility, and this process may be repeated many times, with better reproduction. But there is partial loss of reversibility in Fig. 4 curve b. This could be related to the formation of new crosslinks such as hydrogen bonds or hydrophobic interactions, essentially involving the –COOH, $–NH_2$, and $–N(CH_3)_2$ residues. It would be a desirable characteristic for a pH-sensitive controlled release system with controllable swelling ability.

Temperature dependence of swelling

The effect of temperature on the equilibrium swelling ratios for semi-IPN10 in pH 6.8 buffer solutions is shown in Fig. 5. It presents significant changes in the swelling ratios of hydrogel that occurred over the temperature range 30 to 50 °C. Owning to the PDMAEMA molecule contains a hydrophilic group $–N(CH_3)_2$ and hydrophobic group (ester, −COO−), the hydrophilic group in the polymer structure will form an intermolecular hydrogen bond with surrounding water at low temperature (below the hydrogel volume phase transition temperature). Hence, water penetrating into hydrogel is in a bound state at low temperature. The water molecule will gain an enthalpy during the increase of temperature, and the hydrophilic group in the PDMAEMA will be turned into an intramolecular hydrogen bond in this

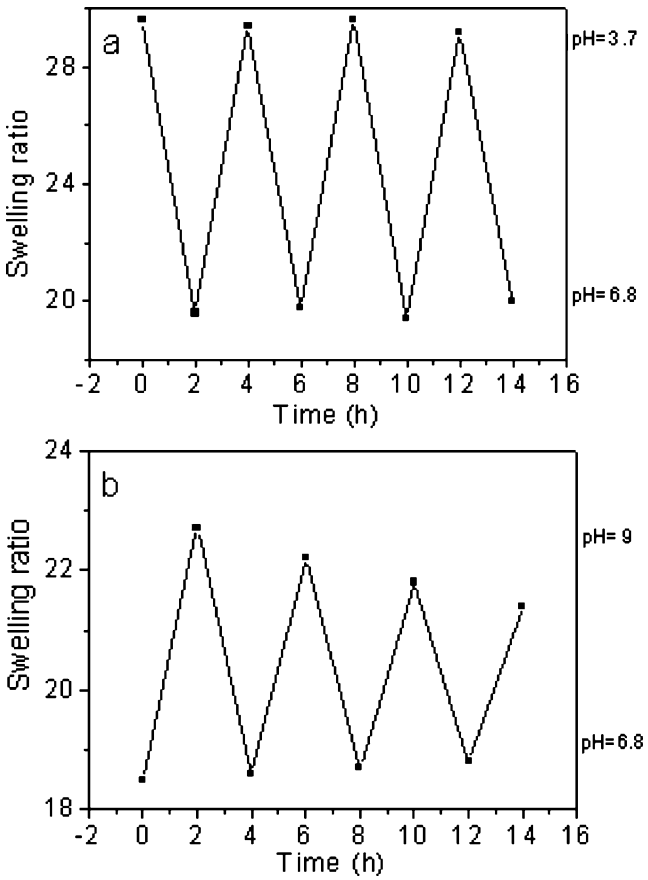

Fig. 4 Swelling and deswelling behavior of semi-IPN10 as a function of time at different pH values solution: **a** pH, 3.7–6.8; **b** pH, 6.8–9

condition. At the same time, the hydrophobic force of PDMAEMA increases. These two results make the water molecule inside the hydrogel change from a bound state to a free state and release from the hydrogel. This phenomenon makes the swelling ratios of the hydrogels decrease rapidly at the hydrogel transition temperature.

The temperature-dependent reversibility behavior of semi-IPN10 is shown in Fig. 6; with the same method used to observe the pH reversibility, semi-IPN10 could swell and shrink with changing temperature between 25 and 50 °C which is similar to the pH sensitivity.

Drug release study

Effect of pH on CoA release

The release profile of CoA from semi-IPN10 (drug loading content: 60 mg of CoA per 1 g dry hydrogel) at various time intervals in pH 3.7, 6.8, and 9 solutions at 25 °C was shown in Fig. 7. There is a burst release initially for the first hour in acidic, neutral, and basic medium, followed by an almost constant release of CoA from the hydrogel for the studied period of 48 h. The initial burst release may be attributed to the release of CoA molecules loaded near the surfaces of the hydrogel. The amount and percentage of CoA release was much higher in acidic than that in basic and neutral solution. The drug in the hydrogel could be released as a result of the hydrogel volume change and effect between the polymer network and CoA. Figure 7 shows that the fractional release is directly proportional to the swelling ratio of the hydrogels, that is, pH=3.7>pH=9 >pH=6.8 (see Fig. 3). This result indicates that the higher swelling ratios of the hydrogel create larger surface areas to diffuse the drug. On the other hand, CoA is an organic acid. With changing the pH of the solution, the charges of CoA were also varied. At pH 3.7 solutions, the pyridine in CoA was positively charged. The $–NH_2$ and $–N(CH_3)_2$ groups in the hydrogel network were all positively charged. The electrostatic repulsion between them accelerates the release of CoA from the hydrogel. At pH 9 alkaline solution, the

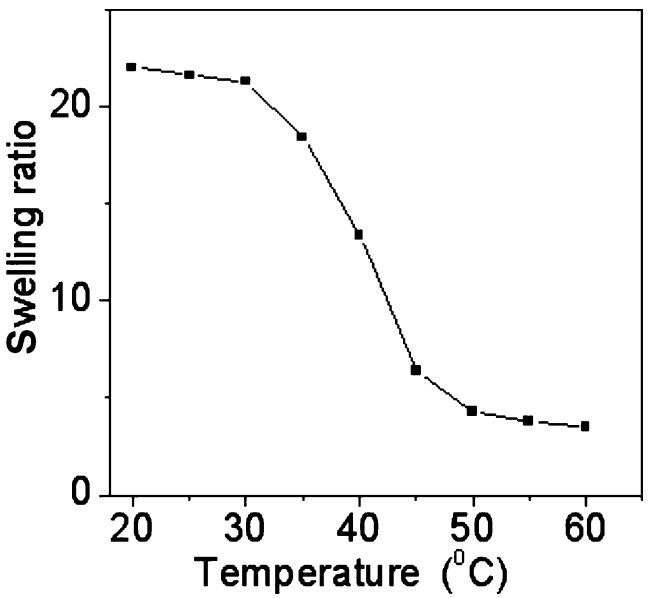

Fig. 5 Effect of temperature on swelling ratio of hydrogel semi-IPN10

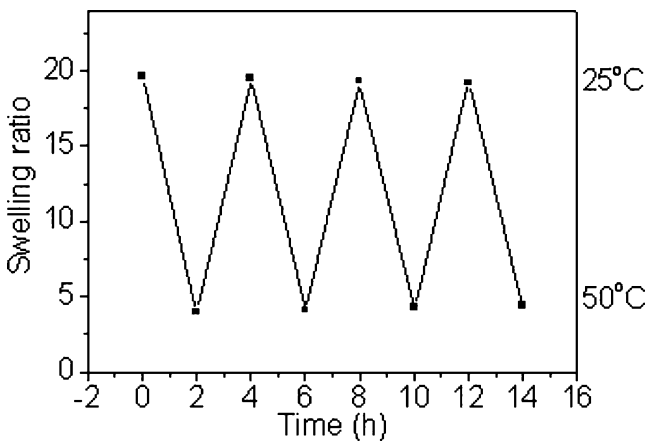

Fig. 6 Swelling and deswelling behavior of semi-IPN10 as a function of time at different temperature under pH 6.8 values solution

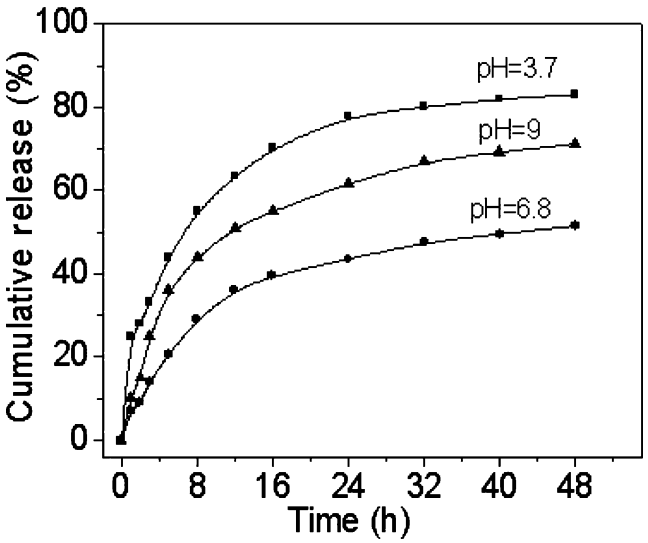

Fig. 7 Effect of pH on release of CoA of semi-IPN10 at 25 °C at different pH buffer solution

phosphoric groups of coenzyme A were negatively charged, and the electrostatic repulsion between phosphoric salt and $–COO^-$ also facilitates the release of CoA. At pH 6.8 solutions, the $–NH_2$, $–N(CH_3)_2$ in the hydrogel network and phosphoric groups, pyridines in the CoA were all partly charged, and the electrostatic attraction between them hindered CoA release from the hydrogel.

Effect of temperature on CoA release

Figure 8 shows the CoA release profiles from semi-IPN10 in a buffer solution at pH=6.8 at different temperature. For semi-IPN10, the highest release rates were obtained at 50 °C while the lowest release rates were observed at 25 °C. Two essential reasons seem to regulate the release behavior. The first is related to the fact that the effective crosslinking density of the CM–CS/PDMAEMA network would be reduced by the precipitation of PDMAEMA, which would accelerate the CoA release [32]. Therefore, the precipitation of PDMAEMA in the hydrogel matrix plays a critical role in squeezing out the entrapped CoA molecules from the hydrogel at 37 and 50 °C. Another reason is that the phosphoric groups and pyridine in the CoA were all partly charged at pH 6.8 solutions, and they were hydrophilic domains of the CoA, and there was electrostatic attraction between them and the function groups in the hydrogel. The substituent of phosphate was relatively hydrophobic part of CoA. At 25 °C below volume phase transition temperature of the semi-IPN10 hydrogel, the substituent of phosphate can form H bond with –OH, $–NH_2$, and $–N(CH_3)_2$ groups in the hydrogel. With the temperature of the release medium increasing, these two effects became weaker, and accelerate the release of CoA from the hydrogel.

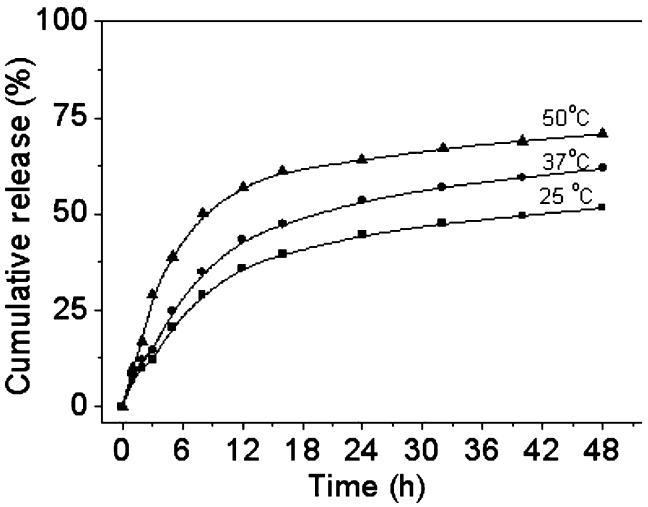

Fig. 8 Effect of temperature on release of CoA of semi-IPN10 at pH 6.8 solution

Effect of CM–CS content on CoA release

Figure 9 presents the effect of CM–CS content on the CoA release behavior at 25 °C at pH=6.8 solution. A tendency for a decrease of CoA release with CM–CS content increasing can be observed. This could also be explained by the term of swelling behavior of semi-IPN hydrogels. Figure 2 shows the SR of the semi-IPN hydrogel of different content of CM–CS. It was found that the hydrogel with 20% CM–CS content showed the minimum SR. For hydrogel delivery systems, the release of CoA is controlled by the swelling behavior of the hydrogel. The swelling of the carrier increases the aqueous solvent content within the polymer matrix, enabling the CoA to diffuse through the swollen network into the external environment.

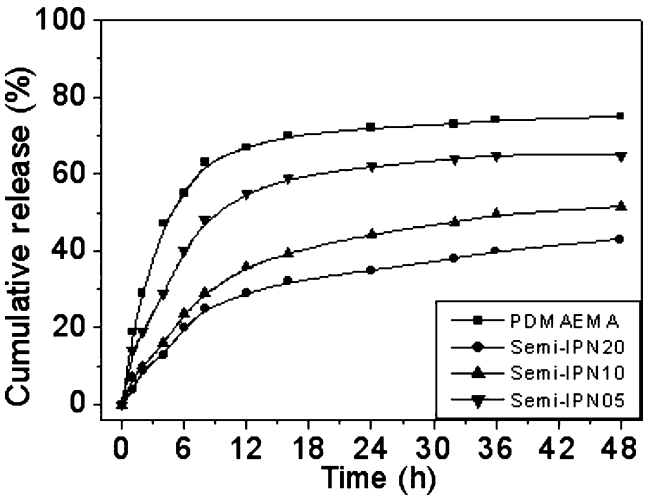

Fig. 9 Effect of CM–CS content on release of CoA at pH 6.8 solution at 25 °C

Conclusions

pH/temperature-responsive semi-IPN polyampholyte hydrogels that were potentially used as drug delivery system based on CM–CS and PDMAEMA were synthesized. The swelling behavior study of the semi-IPN hydrogels demonstrated the pH- and temperature-responsive nature of the materials. The CoA release profile from the hydrogels was also discussed as a function of pH and temperature. The cumulative release rate of CoA was 83.1, 51.5, and 72.2%, respectively, when pH values of drug release medium were 3.7, 6.8, and 9. Furthermore, the release rate of CoA at pH 3.7 solution was the fastest. The release rate was much faster at 50 and 37 °C than that at 25 °C due to the squeezing-out effect of PDMAEMA. At 25 °C and pH 6.8 solution, a tendency for a decrease of CoA release with increasing CM–CS content was also observed. These results suggested that CM–CS/PDMAEMA semi-IPN polyampholyte hydrogels could be used as a pH/temperature-responsive drug delivery system.

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