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Preparation and properties of a pH/temperature-responsive carboxymethyl chitosan/poly(*N*-isopropylacrylamide)semi-IPN hydrogel for oral delivery of drugs

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Abstract—Thermo- and pH-responsive semi-IPN polyampholyte hydrogels were prepared by using carboxymethylchitosan and poly(*N*-isopropylacrylamide) with *N*,*N*′-methylenebisacrylamide (BIS) as the crosslinking agent. The swelling characteristics of these hydrogels at distinct compositions as a function of pH and temperature were investigated. It was found that the semi-IPN hydrogels demonstrated the pH- and temperature-responsive nature of the materials, and it also showed good reversibility. The study on the release of coenzyme A (CoA) showed that within 24 h the cumulative release ratio of CoA was 22.6% in pH 2.1 solution and 89.1% in pH 7.4 solution at 37 °C, respectively. The release rate of CoA was higher at 37 °C than 25 °C in a pH 7.4 buffer solution. An increased release rate of CoA was observed with the content of carboxymethylchitosan increasing in the hydrogel at 25 °C in pH 7.4 solution. These results show that semi-IPN hydrogel seems to be of great promise in pH–temperature oral drug delivery systems.
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Keywords: Biomaterials; Carboxymethylchitosan; Drug delivery systems; pH–temperature-responsive polymer; Semi-IPN polyampholyte hydrogel

1. Introduction

Amphoteric polyelectrolyte hydrogels possessing both positive and negative charges are interesting synthetic analogs for proteins. It is known that materials undergoing continuous or discontinuous volume phase transition responses to solvent composition,[1] pH,[2] salt concentration,[3] temperature,[4] and ultraviolet light[5] have received much attention recently because of their scientific and technological importance.[6–8] 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.[9–12]

It is known that the oral route is the most convenient and comfortable way of administering drugs. Successful oral drug delivery requires that the drug carrier is resistant both to attack by enzymes and to the impact of pH gradients (changing from pH 1–3 in the stomach to pH 6–7 in the intestine) for the gastrointestinal transit time from mouth to caecum (3–16 h) depending on the state of the stomach.[13] Therefore, resistance to acid and enzyme and time-controlled release are necessary for a viable oral drug carrier.

Carboxymethylchitosan (CM-CS), a natural amphoteric polyelectrolyte derived from chitosan has attracted considerable interest in a wide range of biomedical applications, such as wound dressings, artificial bone and skin, bacteriostatic agents, and blood anticoagulants, due to its unique chemical, physical, and biological properties, especially its excellent biocompatibility.[14–16] It has also demonstrated good pH and ion sensitivity in aqueous solutions due to abundant –COOH and $–NH_2$ groups.[17] The high degree of substitution of CM-CS hydrogels will swell significantly in basic solutions and will shrink dramatically in solutions of low-pH. So it is suitable to use as an oral delivery system for drugs.[18]

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Poly(*N*-isopropylacrylamide) (PNIPAm) is a widely studied temperature-sensitive polymer because it has a lower critical solution temperature (LCST) in the range of 30–32 °C, which is near that of the human body (37 °C).[19–21] This thermosensitive polymer has been made for gels[22] and beads,[23] and it has been extensively used as drug delivery systems, bioactive molecule separations,[24] and catalysts.[25] The copolymer of NIPAm with other functional monomers was widely used in the fields of chemistry, materials, and biotechnology.[26]

To combine the advantages of synthetic and natural polymers and at the same time maintain the favorable properties of natural polymers such as biodegradation and bioactivity, amphoteric polyelectrolyte hydrogels with pH- and temperature-sensitivity were synthesized with CM-CS and PNIPAm in this work. The swelling behavior of the semi-IPN hydrogel under different pHs and temperatures was studied. Coenzyme A (CoA) is widely used in the medical area. It can modulate the metabolism of sugar, fat, and protein in the body. It contains $–NH_2$, $–H_2PO_4$, –SH, and –NHCO ionic or polar groups. So the interaction of CoA and the polymer by H-bond or ionic complex occurs easily. CoA is readily soluble in water. In addition, the UV absorbance of CoA takes place at 260 nm, so it is very convenient to monitor it in solution by UV spectroscopy. The release behavior of CoA from the semi-IPN hydrogel was also investigated in simulated gastric and intestinal media in this paper. The results suggest that the hydrogel has great potential in the use of oral drug delivery systems.

2. Experimental

2.1. Materials

Chitosan (CS) was purchased from Tokyo Kasei Kogyo CO., Ltd. The degree of deacetylation (DA) was 0.85 as measured by elemental analysis.[27] Carboxymethylchitosan with 1.08 degree of substitution (DS) as determined by potentiometric titration[28] was prepared according to the literature method.[18] PNIPAm and coenzyme A (CoA) were all purchased from Aldrich Chemical Co. *N*,*N*′-Methylenebisacrylamide (BIS) for use as a cross-linking agent was purchased from Shanghai Reagent Corporation. Ammonium persulfate (APS) was obtained from Peking Chemical Industry, China, and recrystallized before use. All other chemicals were of analytical grade, used without further purification.

2.2. Preparation of CM-CS/PNIPAm semi-IPN hydrogels

Various ratios of NIPAm, CM-CS, and 5 wt % BIS based on the total monomers were dissolved in 6 mL of deionized water as described in Table 1. After bubbling N_2 gas for 30 min to deoxygenate the solution, 1 wt % APS as a redox initiator was added to the solution. Then the mixture was incubated at 40 °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. Then the dry hydrogel was weighed, and it was found that the dry hydrogel weight was almost equal to the materials (CM-CS, NIPAm, and BIS). The thickness of the dried hydrogel was about 1–1.5 mm, and the diameter of the particles 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 a ratio of 10% (w/w) (relative to the total weights of CM-CS and NIPAm), and then gently stirred for 1 h at room temperature before APS was added into the mixed solution.

2.3. Characterization

IR spectra of the hydrogel were recorded using KBr pellets on an AVATAR-360 FTIR instrument at a resolution of 4 cm^{-1}. All the UV spectra of the release medium were recorded with a UV–vis spectrophotometer (UV-540, Vertex Instrument Corp., US).

2.4. 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

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

Component	Sample code			
	PNIPAm	Semi-IPN05	Semi-IPN15	Semi-IPN30
NIPAm (g)	0.480	0.480	0.480	0.480
W (CM-CS) (%)[a]	0	5	15	30
m (BIS) (g)	0.0240	0.0240	0.0240	0.0240
m (APS) (g)	0.0048	0.0048	0.0048	0.0048
V (H_2O) (mL)	6	6	6	6

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

the aqueous solution, after the removal of excess surface water with a filter paper, weighed, and returned to the same container until equilibrium was observed. The SR is 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 a report in the literature.[29] Shortly thereafter, the pH of the solution was adjusted to 2.1 and 7.4 by adding some HCl or NaOH, then adding appropriate amounts of NaCl to the solution to make the ionic strength of the buffers the same.

2.5. Swelling–deswelling–reswelling measurements

Swelling–deswelling–reswelling cycles of semi-IPN hydrogels were carried out at pH 2.1–7.4 or at temperatures around the volume phase transition temperature (VPTT) of the hydrogels. The hydrogels were immersed in solution at 25 °C and pH 7.4 for 2 h, afterwards in solutions at 37 °C for 2 h, and then again immersed in solutions at 25 °C for 2 h. 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 solution pH was done by the same method.

2.6. In vitro drug release

The release rate experiments were performed in a glass apparatus at 25 °C, and at 37 °C under unstirred conditions, or in acidic (pH 2.1), and alkaline (pH 7.4) solutions, respectively. The hydrogel (0.1 g) containing a known amount of CoA was added to the release medium (100 mL). At a given time interval, 1-mL samples were withdrawn and assayed for the amount of released CoA as a function of time. The amount of released CoA was determined by UV spectroscopy at 260 nm using a calibration curve constructed from a series of CoA solutions with standard concentrations. The results are expressed as cumulative release ratio (amount of released CoA/ all amount of loaded CoA). The experiments were done in triplicate.

3. Results and discussion

3.1. FTIR spectrum of the semi-IPN hydrogel

The FTIR spectra of CS, CM-CS, and semi-IPN hydrogel CM-CS/PNIPAm were shown as curves a, b, and c in Figure 1. Curve a shows signals of non-modified chitosan at 1647 and 1590 cm^{-1} for the C–O stretching (amide) and N–H bending (amine), respectively. The

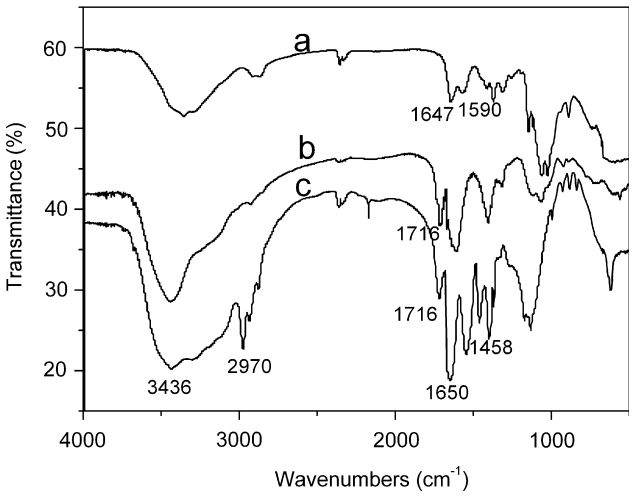

Figure 1. FTIR spectrum of (a) CS, (b) CM-CS, and (c) semi-IPN hydrogel.

spectrum of CM-CS (curve b) is similar to that of the original chitosan (curve a), while a new peak appears at 1718 cm^{-1}, which is assigned to the carbonyl groups on the side chains. Compared to the FTIR spectrum of CM-CS (b), curve c has a new peak appearing around 1650 cm^{-1} corresponding to the –NHCO groups in PNIPAm. The FTIR spectrum of semi-IPN hydrogel (c) also reveals a new peak at 2970 cm^{-1}, which is assigned to C–N in PNIPAm. In addition, the peak intensity at 1458 cm^{-1} attributed to $-CH_3$ is also strengthened. All the above characterization data show that we synthesized the semi-IPN hydrogel.

3.2. Temperature-sensitivity of the semi-IPN hydrogels

The SR of the semi-IPN hydrogels at pH 2.1 and 7.4 in solution as a function of temperature are shown in Figures 2 and 3, respectively. It was found that the SR of the hydrogels decreased with the temperature increasing both at pH 2.1 and 7.4. This is attributed to the collapse of the PNIPAm chains at 37 °C, leading the hydrogel to assume a more hydrophobic state.[30] Owing to the fact that the PNIPAm molecule contains a hydrophilic group, –NHCO, and a hydrophobic group, $-CH(CH_3)_2$, the hydrophilic group in the polymer structure will form an intermolecular hydrogen bond with the surrounding water at low temperature (below the hydrogel volume phase transition temperature). Hence, water penetrating into the 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 PNIPAm will be turned into an intramolecular hydrogen bond under these conditions. At the same time, the hydrophobic force of PNIPAm increases. As a consequence, these two results make the water molecules inside the hydrogel change from a bound state to a free

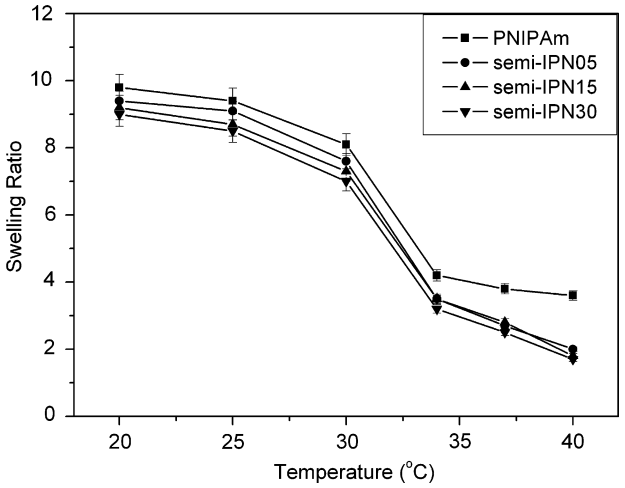

Figure 2. Temperature-dependent changes of the swelling ratio for semi-IPN hydrogels with different compositions in solution at pH 2.1.

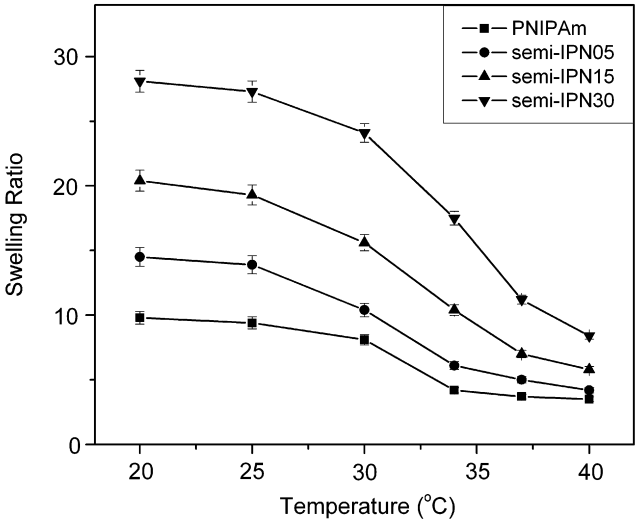

Figure 3. Temperature-dependent changes of the swelling ratio for semi-IPN hydrogels with different compositions in solution at pH 7.4.

state, with subsequent release from the hydrogel. This phenomenon makes the swelling ratios of the hydrogels decrease rapidly at the hydrogel transition temperature.

The sensitivity of the volume phase transition of the hydrogel is also different as shown in Figures 2 and 3. The volume phase transition of the hydrogels in basic solution is more sensitive than that in acidic solution. However, in the experimental condition, the hydrophilicity of the segment of PNIPAm is dominant, both in pH 2.1 and 7.4 solutions, and the heat shrinking characteristic of the semi-IPN hydrogel is observed throughout the experiment.

Since the semi-IPN hydrogel swell differently at different solution temperatures, we have investigated their temperature-dependent swelling reversibility. The swelling reversibility of the semi-IPN15 at 25 and 37 °C are

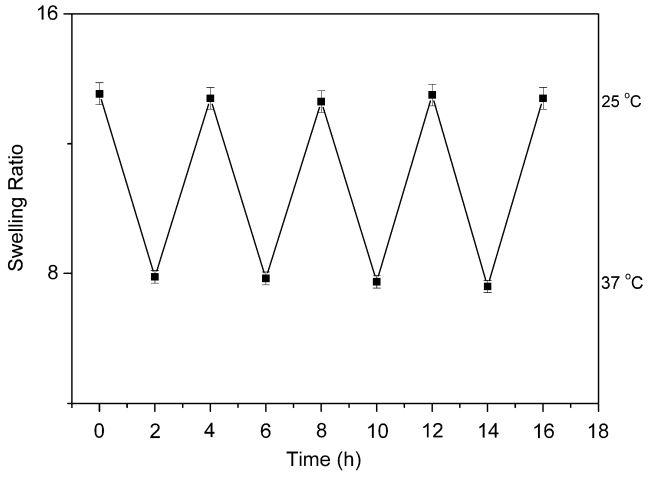

Figure 4. Swelling and deswelling behavior of semi-IPN15 as a function of time at different temperatures in solution at pH 7.4.

shown in Figure 4. It demonstrates the semi-IPN hydrogel reversibility to absorb and deabsorb water upon changing the temperature. The results show that the swelling–deswelling behavior of the semi-IPN hydrogel shows good reversibility, and this process may be repeated many times, with better reproduction. Because the time interval between each step is 2 h, the experimental data in Figure 4 are not equilibrium values. However, the data show that the largest changes in the swelling and deswelling behavior already occurs in the 2 h, especially in the deswelling process of the hydrogel. This may be because CM-CS is acting as a channel for water in the semi-IPN hydrogel, so the water molecules can diffuse easily into or out of the polymer network.

3.3. pH-sensitivity of the semi-IPN hydrogel

The SRs of the semi-IPN hydrogels at 25 °C are between 14 and 28 in pH 7.4 solution (Fig. 3), and are much bigger than that in pH 2.1 solution (Fig. 2) which is about 9. This can be explained by the fact that –COOH can form an H-bond between the –OH groups in the CM-CS and the –NHCO in PNIPAm in acidic solution. Although $–NH_2$ is positively charged in pH 2.1 solution, there is electrostatic repulsion in the polymer network. But the degree of substitution of CM-CS is very high (1.08), and the amount of residual $–NH_2$ is rather limited, so the H-bond is dominant in the polymer network system. A great deal of H-bond between the inter- and intra-molecules make the hydrogel shrink. At pH 7.4 –COOH is negatively charged. The H-bond between –COOH and –OH, –NHCO groups is dissociated, and the electrostatic repulsion between the $–COO^-$ groups makes the hydrogel dramatically swell.

In Figure 2, it is shown that the SR of PNIPAm is always larger than that of the semi-IPN hydrogels in acid solution, and the SR of PNIPAm is always smaller

than that of the semi-IPN hydrogels in basic solution in Figure 3. We also found that the difference of the SR of the hydrogels at 25 °C in pH 2.1 solution was rather small, and in basic solution the difference was much bigger. The SR of the semi-IPN hydrogel increased with the CM-CS content increasing in pH 7.4 solutions as shown in Figure 3. This may also be attributed to the electrostatic force and H-bond between the polymer networks under different experimental conditions.

We also studied the pH-dependent swelling reversibility of semi-IPN15 using the same method that was used to observe the temperature reversibility, and the results are shown in Figure 5. It was found that semi-IPN15 could swell and shrink with changing pH between pH 2.1 and 7.4, which was similar to the temperature-sensitivity. But there is a partial loss of reversibility in pH 2.1 solution. This could be related to the formation of new crosslinks such as hydrogen bonds or hydrophobic interactions,[31] especially involving the –COOH, –OH, and –NHCO groups. It would be a desirable characteristic for a pH-sensitive controlled release system with controllable swelling ability.

3.4. Drug release studies

3.4.1. Effect of temperature on CoA release. Figure 6 shows the CoA release profiles from semi-IPN15 in a buffer solution at pH 7.4 at different temperatures. For semi-IPN15, the higher release rate is obtained at 37 °C, while the lower release rate is observed at 25 °C. Two essential reasons seem to regulate the release behavior. The first is related to the fact that the PNIPAm chains are water solvated and randomly distributed at temperatures below LCST but become collapsed near or above the LCST. Thus, the PNIPAm chains become more hydrophobic, so the effective crosslinking density of the CM-CS/PNIPAm network would

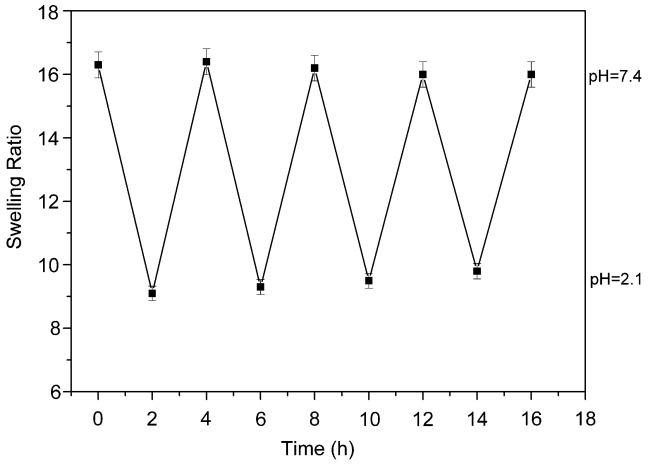

Figure 5. Swelling and deswelling behavior of semi-IPN15 as a function of time at different pH values in solution at 25 °C.

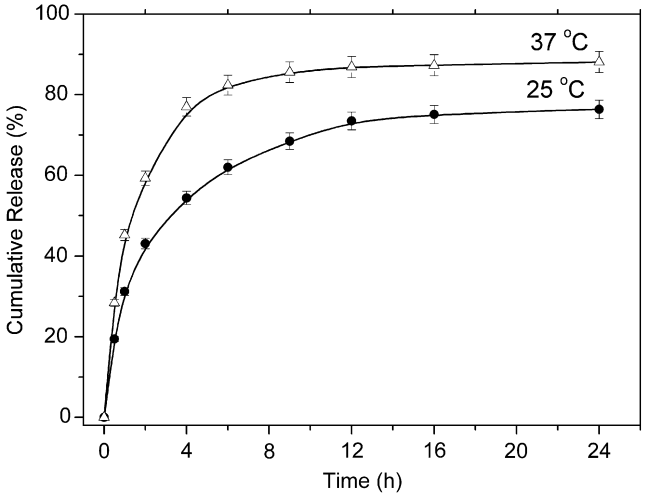

Figure 6. Effect of temperature on the release of CoA from semi-IPN15 in solution at pH 7.4.

be reduced by the precipitation of PNIPAm, so the porous size of the hydrogel was enlarged by the collapsing of PNIPAm chains inside the semi-IPN hydrogel,[32] which would accelerate the CoA release.[33] Therefore, the precipitation of PNIPAm in the hydrogel matrix plays a critical role in squeezing out the entrapped CoA molecules from the hydrogel at 37 °C. Another reason is that at pH 7.4 solutions, there is an electrostatic repulsion between phosphate in CoA and $–COO^-$ in the hydrogel, and it accelerates CoA release. At 25 °C below the volume phase transition temperature of the semi-IPN15 hydrogel, the substitute of phosphate in CoA can form an H-bond with the –OH, –NHCO, and $–NH_2$ groups in the hydrogel. With the temperature of the release medium increasing, the H-bond becomes weaker, and accelerates the release of CoA from the hydrogel. As a result, the release rate in solution at 37 °C is faster than that at 25 °C.

3.4.2. Effect of pH on CoA release. Figure 7 shows the release profile of CoA from semi-IPN15 at various time intervals in solutions at pH 2.1 and 7.4 and 37 °C. There is a burst release initially for the first hour both in acidic and basic medium, followed by an almost constant release of CoA from the hydrogel for the studied period of 24 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 is much higher in basic than that in acidic solution. The drug in the hydrogel could be released as a result of the hydrogel volume change and interaction 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 7.4 > pH 2.1 (See Figs. 2 and 3) in solution at 37 °C. This result indicates that the higher swelling ratios of the hydrogel

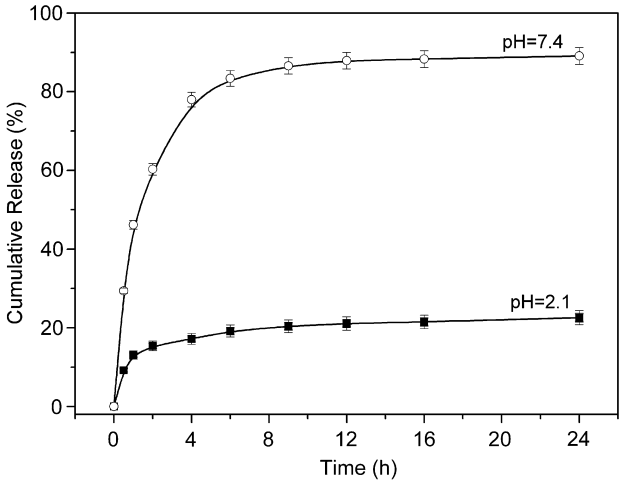

Figure 7. Effect of pH on release of CoA of semi-IPN15 in solution at 37 °C with different pH buffers.

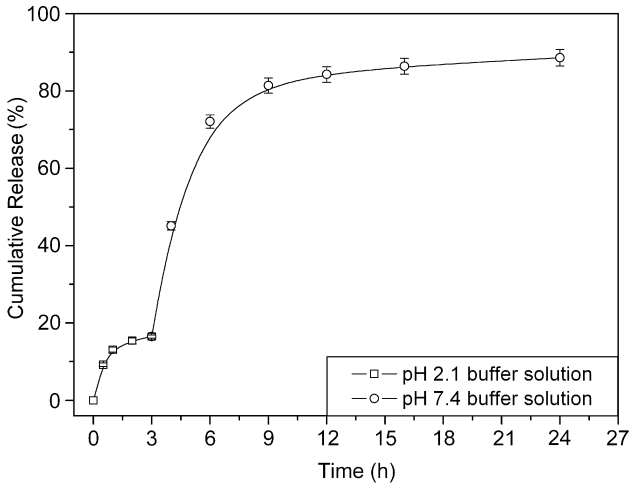

Figure 8. Release profile of CoA from semi-IPN15 in pH 2.1 buffer solution for 3 h with a shift to pH 7.4 for 24 h at 37 °C.

create larger surface areas to diffuse the drug. On the other hand, CoA is an organic acid. With the changing of the pH of the solution, the charges of CoA are also varied. In solutions at pH 2.1, the pyridyl group in CoA is positively charged. The few residual $–NH_2$ groups in the hydrogel network are also positively charged. The electrostatic repulsion between them accelerates the release of CoA from the hydrogel. But a large number of H-bonds between the polar groups in CoA such as –OH, $–NH_2$, $–H_2PO_4$, –SH, and –NHCO and the groups in the polymer network serve to hinder the CoA release from the hydrogel. As a result of the two effects, the release of CoA from the hydrogel at pH 2.1 solution is very low. At pH 7.4 alkaline solution, the phosphoric groups in CoA are negatively charged, and the electrostatic repulsion between phosphoric salt and $–COO^-$ facilitates the release of CoA.

In order to simulate the sequential release profile, CoA-loaded semi-IPN15 hydrogel is suspended in buffer at pH 2.1 for 3 h and then shifted into buffer at pH 7.4, and the results are shown in Figure 8. These indicate that the CoA released from the hydrogel is 16.5% within 3 h in simulated gastric fluid (pH 2.1), and the release rate in simulated intestinal fluid (pH 7.4) reaches nearly 81.4% of the initial drug content 6 h after changing the medium. This implies that this polymer has the desired protective effect for oral delivery of the drug, as a significant fraction of the drug remains in the polymer as the hydrogels pass through the low-pH environment of the stomach. When the hydrogels are transferred to the higher pH solution, a great deal of CoA is released from the polymer system. Thus, in a more practical point of view, these biocompatible hydrogel systems can bypass the acidity of gastric fluid without liberating substantial amounts of the loaded drug. After 24 h, the cumulative CoA released is approximately 88.6%. This is because some CoA molecules may be entrapped within the hydrogel network, and these cannot be released unless the polymer matrixes are degraded.

3.4.3. Effect of CM-CS content on CoA release. Figure 9 shows the effect of CM-CS content on the CoA release behavior in solution at 25 °C and pH 7.4. A tendency for an increase of CoA release with CM-CS content increasing can be observed. This could also be explained in terms of the swelling behavior of semi-IPN hydrogels. It is found that the PNIPAm hydrogel shows the minimum SR, and the SR increases with the CM-CS content increasing (see Fig. 3). 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. Another reason is

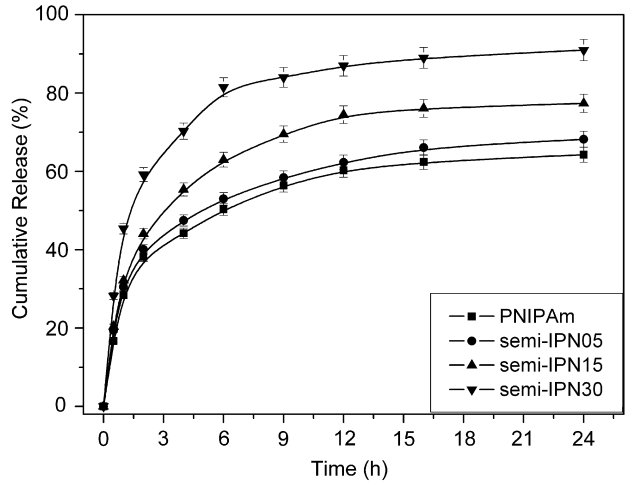

Figure 9. Effect of CM-CS content on release of CoA in solution at pH 7.4 at 25 °C.

that the electrostatic repulsion between phosphate in CoA and $-COO^-$ also increases with the CM-CS content increasing in the hydrogel, and the electrostatic repulsion accelerates CoA release from the hydrogel.

4. Conclusions

In this paper, a semi-IPN hydrogel based on CM-CS and PNIPAm was synthesized. Its swelling behavior showed better temperature- and pH-sensitivity, and good reversibility in solution at different temperatures and pH. The CoA release from the hydrogel was affected by temperature, pH, and CM-CS content in the hydrogel. All the results indicated that this semi-IPN hydrogel can be used as a pH–temperature-responsive orally administered drug delivery system.

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