Preparation, Characterization, and Drug-Release Properties of pH/Temperature-Responsive Poly(*N*-isopropylacrylamide)/Chitosan Semi-IPN Hydrogel Particles

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Received 3 November 2008; accepted 8 October 2009
DOI 10.1002/app.31583
Published online 23 December 2009 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Novel poly(*N*-isopropylacrylamide) (PNIPAAm)/chitosan (CS) semi-interpenetrating polymer network hydrogel particles were prepared by inverse suspension polymerization. The prepared particles were sensitive to both temperature and pH, and they had good reversibility in solution at different temperatures and pH values. The swelling ratios of PNIPAAm/CS hydrogel particles decreased slightly with the addition of CS, which did not shift the lower critical solution temperature. The drug-release behavior of the particles was investigated using cyclic adenosine 3′,5′-monophosphate (cAMP) as a model drug. The release of cAMP from the hydrogel particles was affected by temperature, pH, and the CS content in the particles. These results showed that semi-IPN hydrogel particles appeared to be of great promise in pH- and temperature-sensitive oral drug release. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 116: 1342–1347, 2010

Key words: drug release systems; hydrogels; semi-interpenetrating networks (semi-IPN); stimuli-sensitive polymers

INTRODUCTION

Much research has been focused on stimuli-responsive hydrogel systems which show a phase transition in response to external stimuli, such as, temperature, pH, specific ions, and electric fields.[1–3]

Stimuli-responsive hydrogels have been used in various biomedical and pharmaceutical applications over the past few decades.[4] They have also been extensively studied for use in drug delivery, tissue engineering, and enzyme or protein modification.[5–7] Among these hydrogel systems, pH- or temperature-sensitive 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*.[8–10]

Poly(*N*-isopropylacrylamide) (PNIPAAm) is widely used to prepare temperature-sensitive hydrogels, which show a transition from the swollen state to the collapsed state at temperatures above the lower critical solution temperature (LCST, 32–34°C). This reversible phase transition makes the hydrogel particularly useful for biomedical and bioengineering applications, such as, molecular separations, drug delivery, tissue engineering, and enzyme immobilization.[11]

Chitosan (CS) is the *N*-deacetylation product of chitin, which has long been known for its gel forming ability and has been extensively studied as a physical gel.[12–14] CS has been widely investigated for pharmaceutical purposes owing to its many useful characteristics, such as, low toxicity, high biocompatibility, thickening and film-forming ability, bioadhesiveness, and excellent processability when incorporated into different pharmaceutical systems, which makes CS a novel drug carrier candidate.[15,16]

Many studies concerning PNIPAAm/CS dual responsive hydrogels have been reported. Lee and Chen[17] prepared PNIPAAm/CS semi-IPN and IPN gels by solution polymerization for caffeine release and diffusion. Verestiuc et al.[18] synthesized PNIPAAm/CS gels by radical-induced polymerization acting as a controlled release vehicle for pilocarpine hydrochloride. Carmen et al.[19] also prepared them by free radical polymerzation for diclofenac sodium release. All above methods get the block gels, which swell and shrink slowly, and suitable for drug delivery. Sometimes the gel particles are required for rapid drug release, which have little attentions.

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Contract grant sponsor: National High-Tech Research and Development Plan of China; contract grant numbers: 2007AA021603, 2007AA100404.

In this article, we reported on the synthesis and characterization of semi-interpenetrating polymer networks (semi-IPNs) particles obtained by the inverse suspension polymerization of *N*-isopropylacrylamide (NIPAAm) in the presence of CS. *N*,*N*′-methylenebisacrylamide (BIS) was used as the crosslinker. The swelling behavior of the semi-IPN hydrogel under different pH conditions and temperatures were also studied. In addition, the influence of the CS content to the PNIPAAm content on the swelling characteristics of the hydrogel was considered. Cyclic adenosine 3′,5′-monophosphate (cAMP) was used as a model drug to perform drug release tests. The ability of the system to act as a controlled release vehicle for cAMP was also examined.

EXPERIMENTAL

Materials

NIPAAm and cAMP were purchased from Aldrich Chemical Company (USA). NIPAAm was further purified with benzene/*n*-hexane by recrystallization before use. BIS (Fluka Chemie AG, Switzerland) was used as a crosslinking agent without further purification. Ammonium persulfate (APS), Shanghai Chemical (China), was used as an initiator and was purified by recrystallization in methanol. *N*,*N*,*N*′,*N*′- tetramethylethylene diamine (TEMED), Sigma (USA), was used as an accelerator as received. CS (M_w 400,000) was purchased from Fluka Chemie AG. Acetic acid was purchased from EM Science Industries (Gibbstown, NJ). Sorbitan monopalmitate 40 (span 40) was purchased from Shanghai Chemical, China. All other chemicals were of analytical grade and were used without further purification.

Synthesis of semi-IPN hydrogel particles

Semi-IPN hydrogel particles composed of NIPAAm and CS were prepared by inverse suspension polymerization using BIS as the crosslinker. Cyclohexane (150 mL) was charged to a 500 mL three-necked flask equipped with a mechanical stirrer, condenser, and thermometer. Span 40 (0.4 g) was added as a stabilizer. Nitrogen gas was passed through the solution for 30 min to deoxygenate the solution. Various ratios of CS, NIPAAm, and BIS were dissolved in 6 mL of 0.5 mol/L acetic acid solution as described in Table I. APS (0.05 g) and TEMED (0.1 mL) were introduced to initiate the reaction. The mixture was promptly poured into the experimental flask at a stirring speed of 250 rpm at 20°C. The experiment was kept constant for 6 h, until completion of the reaction. The product was washed successively with ethanol and distilled water. The swollen semi-IPN hydrogel particles were dried in a vacuum oven for 3 days at 30°C to a constant weight.

TABLE I
Feed Composition for the Preparation of Semi-IPN Hydrogel Particles

Component	NC-0	NC-1	NC-3	NC-5	NC-10
NIPAAm (g)	1	1	1	1	1
CS (g)	0	0.01	0.03	0.05	0.1
BIS (g)	0.03	0.03	0.03	0.03	0.03

Scanning electron microscopy

The morphology of the particles was examined by scanning electron microscopy (SEM) (Leica Cambridge S 360) at an accelerated voltage of 15 kV. Before being observed by SEM, the particles were gold coated using an Emscope SC-500 instrument.

Fourier transform infrared spectroscopy

To confirm the successful synthesis of the hydrogels, the chemical structures were characterized by studying their infrared absorption bands using a Fourier transform infrared (FTIR) spectroscope (Thermo Nicolet Avetar 370, USA). FTIR spectra were recorded in the wave number range 4000–500 cm^{-1}.

Swelling ratio measurement

The dried gels were immersed in deionized water at different temperatures or in various buffer solutions of different pH values, until swelling equilibrium was attained. The weight of the swollen hydrogel (W_s) was determined after removing the surface water by blotting with filter paper. The weight of the dried hydrogel (W_d) was determined after drying the gel in a vacuum for 3 days. The swelling ratio (SR) based on W_s and W_d was then calculated from the following equation:

$$\text{Swelling ratio (SR)} = (W_s - W_d)/W_d \qquad (1)$$

Reversibility of swelling ratio measurement

Swelling–deswelling–reswelling cycles of the semi-IPN hydrogels were carried out at pH 2.1 or 7.4 or at temperatures around the LCST of the hydrogels. The hydrogel particles were immersed in solutions at 20°C and pH 7.4 for 20 min, then in solutions at 37°C for 20 min, and then immersed in solutions at 20°C for 20 min. This cycle was continuously run. The weights of the swollen hydrogels were recorded before each immersion. The SR values of the cycle-swollen hydrogels were obtained using eq. (1).

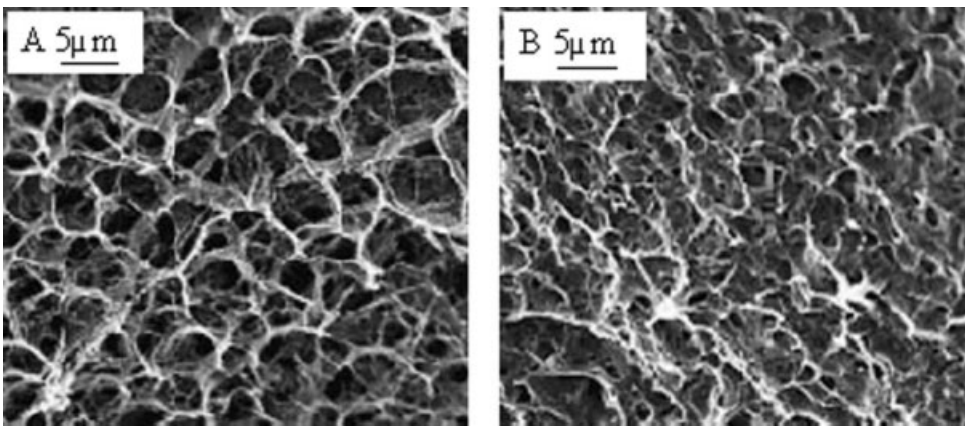

Figure 1 SEM images of hydrogel particles (A) NC-1 and (B) NC-5.

Reversibility measurements at different solution pH were performed at 20°C by the same method.

cAMP release studies

The semi-IPN hydrogel particles were suspended in 50 mg cAMP/10 mL of deionized water at 4°C for 2 days to load the particles with cAMP. The loaded amount of drug was defined as the total amount of cAMP (50 mg) minus the amount of cAMP remained in the deionized water. The swollen and loaded particles containing a known amount of cAMP were placed into the release medium at 37°C or 20°C, or in acidic (pH 2.1), or alkaline (pH 7.4) release media. Samples (1 mL) were periodically removed and the withdrawn sample was replaced by the same volume of fresh medium. The amount of cAMP was determined by UV spectroscopy (JASCO V-530) at 254 nm. The results were expressed as cumulative release ratio (amount of released cAMP/total loaded cAMP).

RESULTS AND DISCUSSION

Characterization of the particles

Figure 1(A,B) showed the SEM images of the semi-IPN hydrogels of NC-1 and NC-5, respectively. The images clearly showed significant 3D porous structure, and the pore size of the hydrogel particles was reduced with the increase of the CS content, whereas the number of pores was greatly increased.

The FTIR spectra of CS and the semi-IPN hydrogels were shown as curves A and B, respectively, in Figure 2. Curve A showed a signal for CS at 1647 cm^{-1} and 1590 cm^{-1} corresponding to C—O stretching (amide) and N—H bending (amine), respectively. The FTIR spectrum of semi-IPN hydrogel (B) also revealed a new peak at 2970 cm^{-1}, which was assigned to the C—N bond in PNIPAAm. In addition, the peak intensity at 1458 cm^{-1} attributed to —CH^3—CH was also strengthened.

Temperature-sensitivity of the semi-IPN hydrogel particles

The effects of temperature on the swelling ratio of the semi-IPN hydrogel particles in deionized water were presented in Figure 3. These results showed that the particles demonstrated LCST at temperatures around 32°C. The introduction of CS into the PNIPAAm network did not change the LCST of the particles. Similar results have been observed with PAAc/PNIPAAm IPN networks interpenetrating polymer networks.[20] This may be explained by the interaction of the CS chain and the PNIPAAm network that gave a relatively independent polymer system, in which each retained its own properties.

It was also found that the introduction of CS led to a slight decrease in the swelling ratio. The formation of semi-IPN restricted the swelling of the hydrogel particles. Therefore, the swelling ratio of the particles decreased with increased CS content.

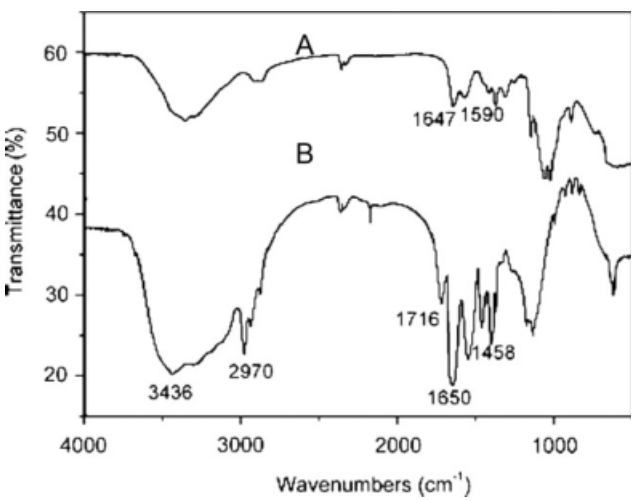

Figure 2 FTIR spectrum of (A) CS and (B) semi-IPN hydrogel NC-5.

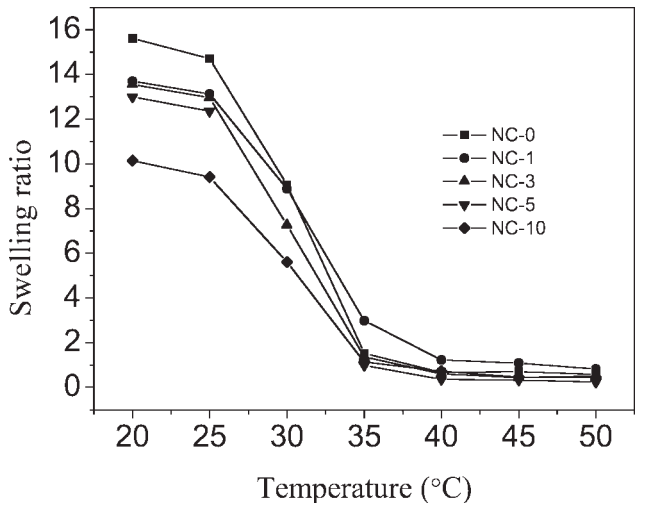

Figure 3 Temperature dependence of the swelling ratio of the hydrogel particles at pH 7.4.

pH-sensitivity of the semi-IPN hydrogel particles

The equilibrium swelling ratios for a series of the semi-IPN hydrogel particles in different pH solutions at 20°C, presented in Figure 4, indicated that the swelling ratios decreased with increasing pH value of the buffer solutions. All particles were seen to exhibit very low water uptake at high pH values. However, when pH decreased, the capable of water uptake of the particles was increased. This was due to protonation of amino groups (—NH_2) in the CS chains and dissociation of hydrogen bonding in acidic solutions, which induced gel swelling and the development of internal ion osmotic pressure. A lower swelling ratio was observed in alkaline solution, which was due to the inherent hydrophobicity of CS.[21,22] Figure 3 also presents that the swelling ratio of the particles decreased with the introduction of CS because the formation of semi-IPN restricted the swelling of the hydrogel particles.

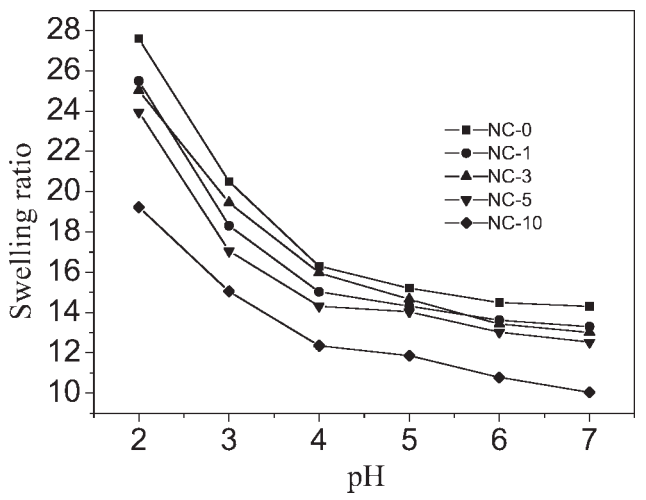

Figure 4 pH dependence of the swelling ratio of the hydrogel particles at 20°C.

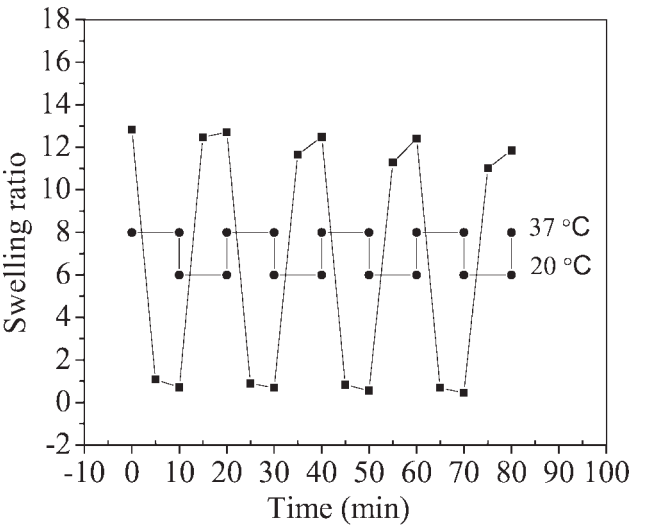

Figure 5 Reversible swelling kinetics of the NC-3 hydrogel particles in response to temperature at pH 7.4.

Reversibility of swelling ratio

Because the semi-IPN hydrogel particles swelled differently under different solution conditions, we investigated their temperature-dependent and pH-dependent swelling reversibility.

Figure 5 presented the effect of temperature change on the particles at pH 7.4. It was found that the particles swelled and deswelled over a period of time when the temperature was periodically varied between 20°C and 37°C. This was reflected in a rapid drop in the swelling ratio when the temperature was changed to 37°C and an increase in the swelling ratio on cooling to 20°C. Figure 6 presents

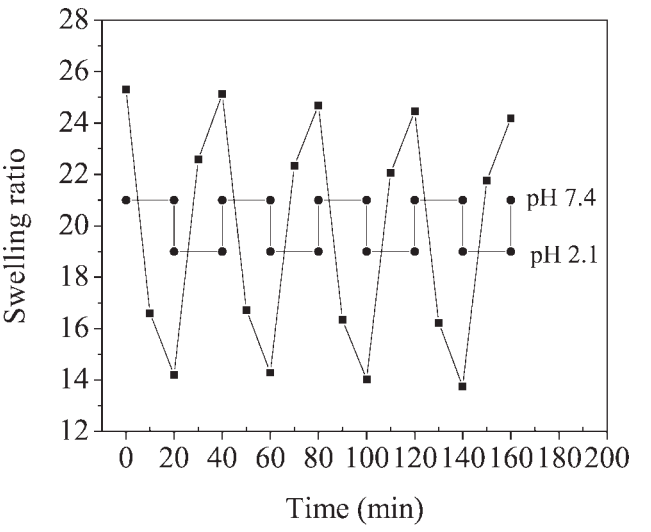

Figure 6 Reversible swelling kinetics of the NC-3 hydrogel particles in response to pH at 20°C.

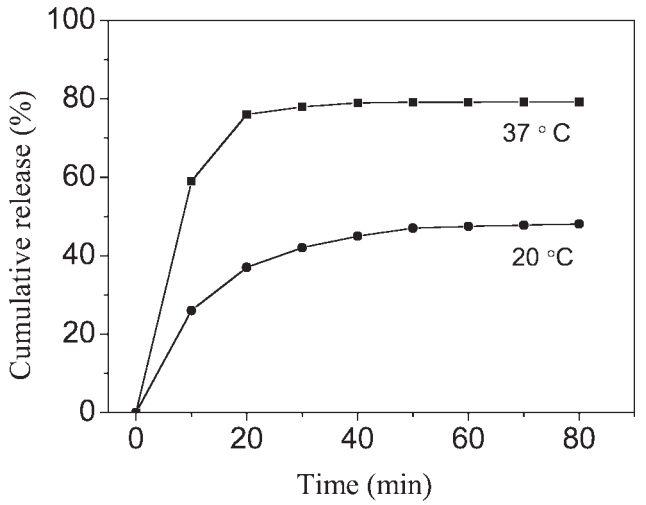

Figure 7 Effect of temperature on the release of cAMP from NC-3 at pH 7.4.

the effect of periodic change in pH on the particles at 20°C. The pH of the buffer solution was changed from 2.1 to 7.4, and the same cycle was repeated. It was also found that the swelling–deswelling cycles were accompanied by a slight decrease in the swelling ratio. This was due to the slower reswelling kinetics of the hydrogel when compared with the deswelling kinetics.[23]

Effect of temperature on cAMP release

Figure 7 presents the release profile of cAMP from NC-3 in a buffer solution of pH 7.4 at different temperatures. For NC-3, the higher release rate was obtained at 37°C, whereas the lower release rate was observed at 20°C. The main reason for the higher release rate at 37°C was due to shrinkage of the particles at 37°C, which released the cAMP in the particles due to the driving force of the volume change and resulted in a drug concentration gradient. Therefore, the precipitation of PNIPAAm above the LCST led to the drug being squeezed out.[24] At temperatures below the LCST, the drug release was governed by diffusion. The surface of the gel immediately shrank forming an impermeable "skin layer," which slowed or prevented further drug release.[23] As a result, the amount of drug in solution at 37°C was higher than that at 20°C

Effect of pH on cAMP release

Figure 8 presents the release rates of cAMP from NC-3 in solutions of pH 2.1 and 7.4 at 37°C. The higher release rate was obtained at pH 7.4, whereas the lower release rate was observed at pH 2.1. The amount of cAMP released was much higher in the basic solution than in the acidic solution. This may be a result of the hydrogel volume change and an interaction between the polymer network and cAMP. The main reason why the release rate was higher at pH 7.4 was similar to that in the temperature experiments, in which the particles shrank at pH 7.4 leading to the drug being squeezed out. In contrast, in solutions of pH 2.1, a large number of H-bonds between the polar groups in cAMP, such as, —OH, —NH_2, —HPO_4 and groups in the polymer network, hindered cAMP release from the hydrogel.

These experiments demonstrated that this polymer had the desired protective effect required for the oral delivery of drugs, as a significant fraction of the drug remained in the polymer as the particles passed through the low-pH environment, similar to that in the stomach. When the particles were transferred to the higher pH solution, a significant amount of the drug was released from the polymer system. Thus, it is considered that these biocompatible hydrogel systems will survive the acidity of gastric fluid without liberating substantial amounts of the loaded drug.

Effect of CS content on cAMP release

Figure 9 presents the effect of CS content on the release of cAMP in solution at 37°C and pH 7.4. When CS content of the particles was increased, cAMP release was decreased. The main reason for this was the pore size of the particles. During the preparation of semi-IPN hydrogel particles, the particles were dried at room temperature, which resulted in them having a porous structure. Thus, when the CS content was high, which decreased the swelling ratio and resulted in particles with smaller

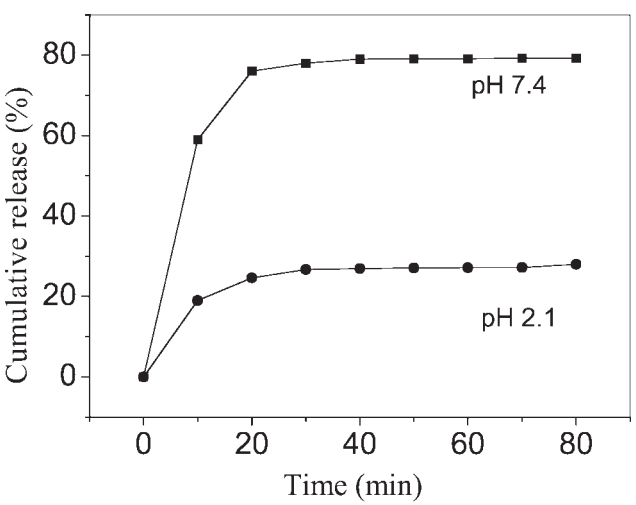

Figure 8 Effect of pH on the release of cAMP from NC-3 at 37°C.

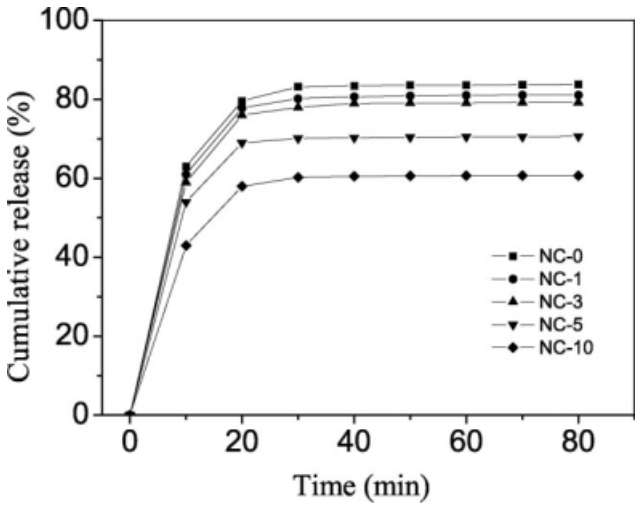

Figure 9 Effect of CS content on release of cAMP at 37°C and pH 7.4.

pores. Therefore, the amount of cAMP released from these particles with smaller pores would be less.

CONCLUSIONS

In this study, PNIPAAm/CS semi-IPN hydrogel particles were prepared by the inverse suspension polymerization method. The swelling behavior of the particles showed good pH- and temperature-sensitivity, and good reversibility in solution at different temperatures and pH values. The swelling ratios of PNIPAAm/CS hydrogel particles decreased slightly with the addition of CS, which did not shift the LCST. The drug-release behavior of the particles was investigated using cAMP as a model drug. The cAMP release from the hydrogel particles was affected by temperature, pH, and the CS content in the hydrogel particles. To conclude, the particles prepared in this study could be considered as potential drug carriers for pH/temperature-responsive orally administered drug delivery systems.

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