Colloid Polym Sci (2008) 286:175–181
DOI 10.1007/s00396-007-1749-y

ORIGINAL CONTRIBUTION

pH and ionic sensitive chitosan/carboxymethyl chitosan IPN complex films for the controlled release of coenzyme A

Bao-lin Guo · Jin-fang Yuan · Qing-yu Gao

Received: 25 October 2006 / Revised: 18 June 2007 / Accepted: 22 July 2007 / Published online: 29 August 2007

Abstract pH and ionic sensitive interpenetrating polymer network (IPN) complex films based on chitosan (CS) and carboxymethyl chitosan (CM-CS) were prepared by using glutaraldehyde as crosslinking agent. Its structure was characterized by FT-IR, which indicated that the IPN was formed. The films were studied by swelling, weight loss with time, and release of coenzyme A (CoA). It was found that the IPN films were sensitive to pH and ionic strength of the medium. The cumulative release rate of CoA decreased with CoA loading content, ionic strength or crosslinking agent increasing. The composition of the IPN films and pH of release medium also had significant effect on the release of CoA. The differences in the rates and amounts of released CoA may be attributed to the swelling behavior, the degradation of films, and interaction between drug molecule and polymer matrix. These results suggested CS/CM-CS IPN films could be used as drug delivery carrier.

Keywords Chitosan · Biomaterials · pH and ionic sensitive · IPN complex films · Drug delivery systems

Introduction

New controlled drug delivery systems in response to changes in environmental conditions, e.g., temperature [1–3], pH [4–6], exposure to ultraviolet [7], visible radiation [8], electric field [9, 10], and in the presence of certain chemicals [11, 12], are being explored. Biodegradable polymers have been used extensively in biomedical areas in the form of sutures, wound covering materials, artificial skin, and for the controlled release of drugs [13, 14]. Natural polymers have become more and more important for their rich resources and low costs, especially for their unique properties, such as nontoxicity, degradability, and good biological compatibility [15]. Among these polymers, chitosan (CS) has been receiving increasing attention in the pharmaceutical field for a wide range of drug delivery applications [16–19]. CS contains a large number of hydroxyl and amino groups, and it can thus be modified by various chemical reactions to prepare a series of CS derivatives as nontoxic biocompatible biomaterials [20–24].

Carboxymethyl chitosan (CM-CS), a natural amphoteric polyelectrolyte derived from CS 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 [25–27]. It has also demonstrated good pH and ion sensitivity in aqueous solution due to abundant –COOH and $–NH_2$ groups [28].

Recently, the use of complexation of oppositely charged macromolecules to prepare CS complexes as controlled drug release formulations, especially for peptide and protein drug delivery, has attracted much attention, as this process is simple, feasible, and can usually be performed under mild conditions [29, 30]. In these systems, it was found that some of the polymer complexes could show pH sensitivity and ion sensitivity due to the dissociation of two components in the complexes. With respect to the excellent film-forming properties of CS, many new and original film materials have been achieved [31, 32]. Drug loaded film is one of the applications for those films in the pharmaceutical technology. In this paper, a series of CS/CM–CS IPN complex films were synthesized by using glutaraldehyde as

B.-l. Guo · J.-f. Yuan · Q.-y. Gao (✉)
Institute of Fine Chemical and Engineering, Henan University, Kaifeng, Henan 475001, People's Republic of China
e-mail: qingyugao@henu.edu.cn

crosslinking agent. CS/CM-CS complex films are also novel film materials and have not previously been reported to be used as drug release carriers. To use these films in several controlled release applications, it is necessary to have an overall understanding of their properties in drug controlled release. Thus, using coenzyme A (CoA) as a model drug, we studied some of the influential factors, which primarily included the component ratio of CS and CM-CS, the loaded amount of CoA, the pH and ionic strength of the release medium, and the crosslinking agent on CS/CM-CS drug loaded films.

Experimental

Materials

Chitosan (CS) was obtained from Tokyo Kasei Kogyo. The degree of deacetylation was 0.85 as measured by elemental analysis [33]. CM-CS with 0.77 degree of substitution as determined by potentiometric titration [34] was prepared according to the literature method [35]. CoA was purchased from Aldrich. Glutaraldehyde was purchased from Chemical Reagent (Shanghai, China). All other chemicals used were of analytical grade, without further purification.

Preparation of drug-loaded complex IPN films

CS/CM-CS drug-loaded films were produced by a casting/solvent evaporation technique. CS was dissolved in a 2% (*w*/*v*) acetic acid water solution to prepare a 4% (*w*/*v*) CS solution, and the 4% (*w*/*v*) CM-CS solution was achieved by dissolving CM-CS in distilled water. These two kinds of solutions were then blended in fixed ratios, from which five kinds of mixed solutions were obtained. The mass ratios of CS to CM-CS of these five blended solutions were 100:0, 70:30, 50:50, 30:70, and 0:100, respectively. CoA (0.2 g) was dissolved in 50 ml of each of these five solutions. The solutions were thoroughly mixed by continuous stirring. Glutaraldehyde, used as crosslinking agent, was added into the blended solution at the amount of 0.01 mole per glucosamine unit of CS. After adding glutaraldehyde, the solutions were sonicated to get rid of air bubbles before casting onto the clean dry Teflon plate at room temperature. The films were dried in a vacuum oven for 3 days at 30 °C to a constant weight. The thickness of the films was kept between 25–30 mm. Thus, a series of CS/CM-CS drug-loaded films with CoA were produced and designated as A1, A2, A3, A4, and A5.

By the above method, 0.1 or 0.3 g CoA was dissolved in the mixed solution of CS and CM-CS, at a mass ratio of CS to CM-CS of 50:50, thus producing drug-loaded films B1 and B2, respectively.

For different concentration of crosslinking agent samples, the 0.2 g CoA loaded blend films with 50% CS content containing glutaraldehyde concentrations of 0.001, 0.05, and 0.5 mole per glucosamine unit were prepared, and they were coded as C1, C2, and C3.

Characterization

The IPN films were immersed in pH 2.1 solution for 12 h, and then, they were dried in vacuum oven. The IR spectra of the vacuum-dried IPN films were recorded using KBr pellets on AVATAR-360FT-IR at a resolution of 4 cm^{-1}.

Swelling and degradation studies

The swelling ratio (SR) was determined by immersing the dry IPN films in aqueous solution of the desired pH or ionic strength 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 a swollen equilibrium was reached. The time for the IPN films to reach equilibrium was 48 h in the tested solution. SR was calculated from the equation $SR = (W_s - W_d)/W_d$, where W_s and W_d represented the weights of the swollen and dry state samples, respectively. The pH of the external solution was adjusted according to literature values [36].

The crosslinked CS/CM-CS IPN complex films were expected to undergo degradation by the hydrolysis of the amino/imine bonds. This process was accompanied with macroscopic changes in the appearance of the films, including changes in the physical mechanical properties of the polymeric films, deformation or structural disintegration, weight loss, and the eventual loss of functions. Hence, hydrolytic degradation [37] of the films (A1–A5 and C1–C3) was studied under physiological conditions. The IPN complex films were placed in 100 ml solution of pH 2.1 and 7.4 at 37 °C under unstirred conditions, and the hydrolytic degradation of the films was determined by the following equation: $\text{weight loss}\,(\%) = (W_o - W_t)/W_o \times 100\%$, where W_o was an initial weight of the films and W_t was a weight of the vacuum-dried films after immersion for 72 h. In these solutions, the concentration of NaCl was 0.01 mol/l.

In vitro drug release

The release experiments were performed in a glass apparatus at 37 °C under unstirred conditions in acidic (pH 2.1) and basic (pH 7.4) solution and under different ionic strength solutions. The release medium of CoA from the films was phosphate buffer solution (PBS, pH=7.4 or 2.1). The ionic strength of the buffer solution was carefully adjusted to a constant level by adding an appropriate

amount of NaCl. The films (0.1 g) containing a known amount of CoA were added to the release medium (100 ml). At a time interval, 1 ml sample was withdrawn and assayed for the amount of released CoA as a function of time. The amount of CoA released was determined by UV spectrophotometer (UV-540, US) at 260 nm using a calibration curve constructed from a series of CoA solution with standard concentrations. The results were expressed as cumulative release (amount of released CoA from the films/all amount of loaded CoA in the films ×100%). The experiments were done in triplicate.

Results and discussion

FTIR spectrum of IPN films

The IR spectrum of CS, CM-CS, and IPN CS/CM-CS film (sample A3) at pH 2.1 values were shown as curves a, b, and c in Fig. 1. Curve a showed signals of non-modified CS at 1,647 and 1,590 cm^{-1} for the C–O stretching (amide) and N–H bending (amine), respectively. The spectrum of CM-CS (curve b) was similar to that of the original CS (curve a), while a new peak appeared at 1,718 cm^{-1}, which was assigned to the carbonyl groups on the side chains. Compared to the IR spectrum of CS (a) and CM-CS (b), curve c had a new peak appearing around 1,635 cm^{-1} corresponding to the formation of the C=N groups by imine reaction between amino groups of CS with aldehydic groups of glutaraldehyde, which demonstrated that the crosslinking reaction occurred in the IPN films. The peak around 1,519 cm^{-1} was attributed to $-NH_3^+$ in pH 2.1 values. In addition, curve c having all the characteristic peaks of curves a and b, the peak corresponding to –OH and $–NH_2$ groups at around 3,418 cm^{-1} became broader, indicating strong hydrogen bond formed in this polymer system. All the above characterization showed that we synthesized the IPN films.

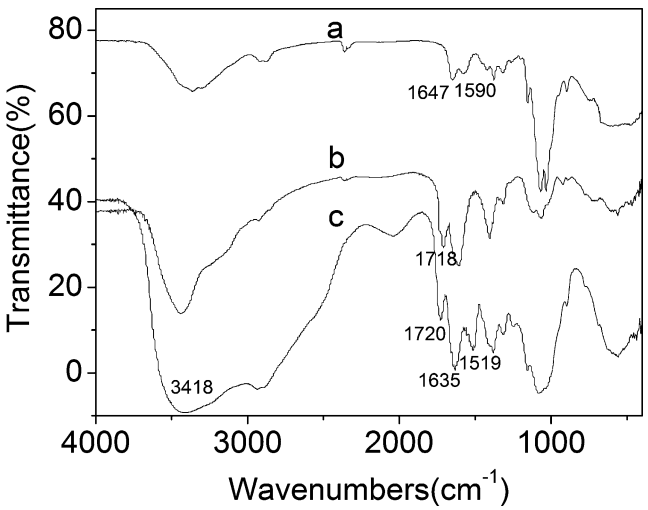

Fig. 1 IR spectrum of CS (**a**), CM-CS (**b**), and sample A3 (**c**) at pH 2.1 value

Effect of crosslinking agent concentration on the SR of the IPN films

Effect of crosslinking agent on the SR for sample A3 and C1–C3 was shown in Table 1. It was found that the SR of the complex films decreased with the glutaraldehyde concentration increasing. The crosslinking density was increased with an increase in glutaraldehyde concentration. The hydrophilicity of the polymer network decreased, and it was difficult for water to invade into the polymer matrix, so the SR decreased. We can tune the glutaraldehyde concentration to prepare IPN films with approximate SR.

Effect of different blend composition on SR at pH 2.1 and 7.4 values solution

Effect of pH on SR at 37 °C for sample A1–A5 was studied in this experiment, and the results were shown in Fig. 2. It was found that at pH 2.1 solution, the highest values of SR were obtained for sample A1, and the SR of the IPN films tended to decrease with the CS content decreasing. In basic medium (pH=7.4), the tendency was opposite. The rule was that the higher the CM-CS content, the higher SR was observed. This can be explained by the electrostatic repulsion and H-bond between the groups in the IPN network. Generally, the swelling process of the film involves protonation and deprotonation of amino/imine and –COOH groups in the film and mechanical relaxation of coiled polymeric chains. Although protonation takes place in a very short time, the overall process is much more complex [38]. The p*K*a of CS is 6.3–6.5, indicating that CS tends to protonate in acidic solution. In an acidic medium (pH=2.1), the amino groups of CS are protonated, resulting in the hydrogen bonds between CS and CM-CS being broken and the network dissociating [35]. The electrostatic repulsion between $-NH_3^+$ makes the films further swell. At alkaline medium (pH=7.4), the –COOH groups are negatively charged, the electrostatic repulsion between $–COO^-$ and decreasing H-bond between –COOH and –OH, $–NH_2$ lead the film swell. So at pH 7.4 solutions, the SR of the films increased with the CM-CS content increasing.

Effect of ionic strength on SR at different pH values solution

Figure 3 shows SR in various aqueous NaCl concentrations for sample A3 at 37 °C. These films were prepared with constant amount of 0.01 mole glutaraldehyde per glucosamine unit of CS. In Fig. 3, it was found that as ionic

Table 1 Influence of crosslinking agent concentration on swelling ratio and percent weight loss of the films at different pH values solution

pH	Swelling ratio				Weight loss (%)[a]			
	Sample code				Sample code			
	C1	A3	C2	C3	C1	A3	C2	C3
2.1	20.2	13.6	12.1	8.0	35.7±3.0	26.4±2.7	21.4±2.3	18.6±2.5
7.4	6.49	5.47	3.14	1.26	19.6±2.1	15.4±1.7	12.2±1.6	10.6±1.8

[a] The average value from three experiments.

strength increased, the gels shrank accordingly. The driving force for the swelling and shrinking of film is the difference between the concentration of free ions inside and outside the film according to the Donnan equilibrium [39]. When the concentration of mobile ions inside the film is lower than that in surrounding condition, the osmotic pressure in the surrounding causes the film to shrink; otherwise, a large osmotic pressure inside the film causes the film to swell. As the concentration of NaCl was increasing, the exterior osmotic pressure increased, and interior osmotic pressure of the IPN films was the same. The exterior osmotic pressure was larger than that in the IPN films, so SR of the IPN films decreased with the concentration of NaCl increasing.

Drug release study

Effect of the amount of drug loaded on CoA release

To study the effect of drug loading content on CoA release, IPN films A3, B1, and B2 with different drug loading content were studied. The pH 2.1 values solutions with the same ionic strength were used as the release medium. From Fig. 4, we can conclude that the release pattern of the higher drug loading content film has been found to be similar to that of the film with a lower loading content of CoA. The cumulative release of CoA from the film is decreased with increasing CoA loading content. However, the total amount of CoA released is found to be more from higher loaded film in comparison to lower loaded films. This result agrees well with the literature [40].

Effect of composition ratio and time on CoA release at pH 2.1 and 7.4 solutions

The effect of blend composition on CoA release at pH 2.1 and 7.4 values solution is shown in Figs. 5 and 6, respectively. Samples A1–A5 were used in this study. It was found that the maximum release of CoA was observed for sample A1, and the lowest release amount of CoA was sample A5. This could be explained by the term of swelling behavior of the IPN films. As shown in Fig. 2, it was found that the CS film showed the maximum SR. It is known that for film delivery systems, the release of the drug is controlled by the swelling behavior of the film. The swelling of the carrier increases the aqueous solvent content within the polymer matrix, enabling the drug to diffuse through the swollen network into the external environment.

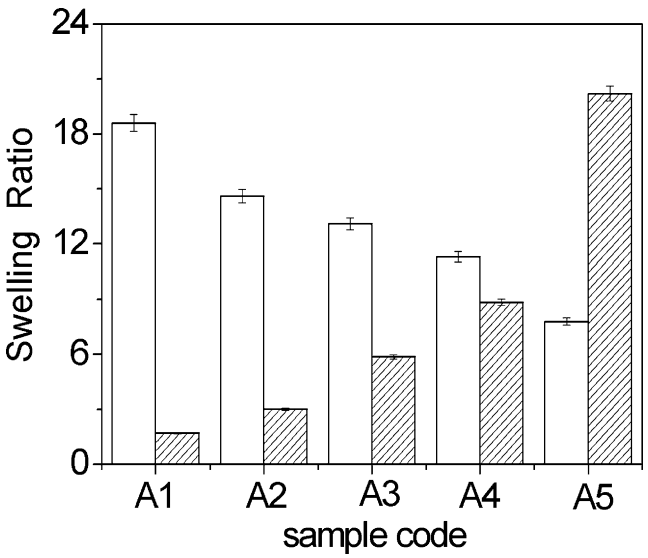

Fig. 2 Influence of pH on SR of different blend composition samples, acid: *blank*, pH 2.1; *hatched*, pH 7.4

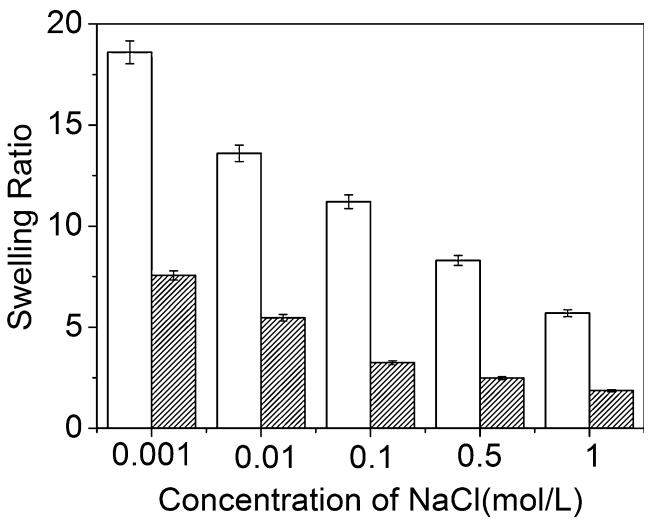

Fig. 3 Influence of ionic strength on SR of sample A3 at different concentration NaCl solution, acid: *blank*, pH 2.1; *hatched*, pH 7.4

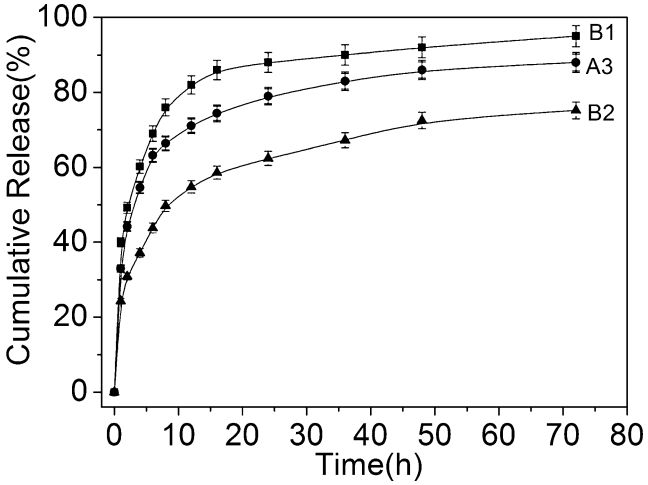

Fig. 4 Influence of the amount of drug loaded on CoA release at pH 2.1values solution

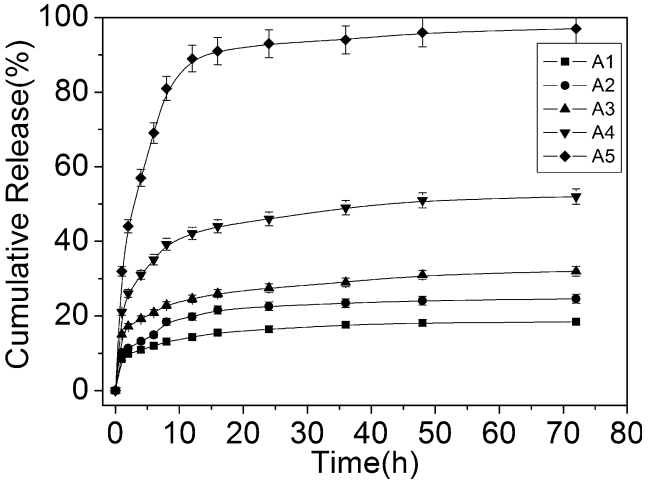

Fig. 6 Effect of composition ratio and time on CoA release at pH 7.4 solution

The pH of the release medium also has significant effect on CoA release. As shown in Figs. 5 and 6, there is a burst release initially for the first hour in both acidic and basic medium, followed by an almost constant release of CoA from the matrix for the studied period of 72 h. It is noticed that CoA release is pH dependent. This can be explained considering the rate of diffusion from the swollen film in acidic and basic solution. For sample A1, A2, A3, and A4 in basic medium (pH 7.4), there is a limited swelling of the film (see Fig. 2), which inhibits the diffusion of drug at a faster rate as it occurs in acidic medium (pH 2.1). Initially, the magnitude of swelling of film in acidic medium is very high and gives rise to a significant burst effect through uncontrolled diffusion but becomes almost constant due to controlled diffusion at film equilibrium swelling. Sample A5 contains more –COOH groups in the network, and SR in alkaline condition (pH 7.4) is bigger than that in acid solution (pH 2.1). Therefore, the release of CoA in pH 7.4 solution from sample A5 is bigger than that in pH 2.1 solution.

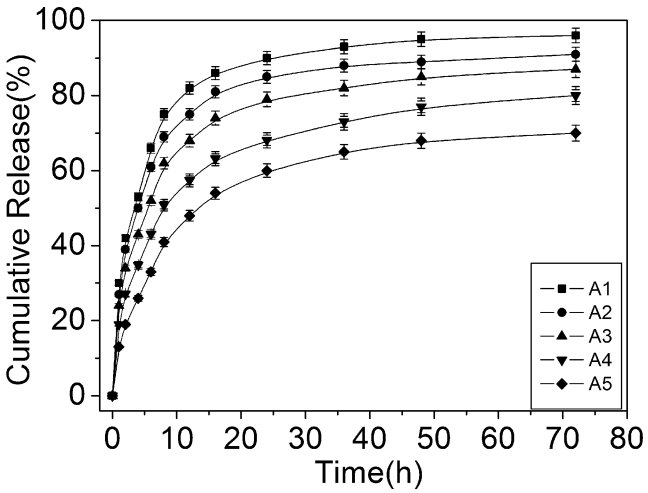

Fig. 5 Effect of composition ratio and time on CoA release at pH 2.1 solution

Besides the release of drug being controlled by the swelling behavior of the carrier, drug release may also be concerned with an erosion process. Table 2 shows the weight losses of the IPN films under the studied conditions. It was found that the weight loss of the films at pH 2.1 solutions was much higher than that in pH 7.4 solutions, and the weight loss of A1 to A5 in pH 2.1 solutions and A5 to A1 in pH 7.4 solutions was decreased. This indicated that drug released by an erosion process could also be occurring in this system.

In Fig. 6, it was also found that the release of CoA from the sample A5 (crosslinking CM-CS film) was very fast and took the shortest time to reach equilibrium as compared with the other samples. It may also relate to the fastest swelling and the biggest SR in all these samples. In Fig. 6, the cumulative release rate of CoA from all samples A1, A2, and A3 were very low, except the limited SR in the pH 7.4 solutions, drug–polymer interaction between CoA and matrix may also play an important role. As CoA has several polar groups, including a hydroxyl group, amino group, and phosphoric group, which can interact with the polymer matrix, so an interaction between CoA and the polymer matrix may occur. As a result, the amount of CoA released from the IPN films was only about 15–30%.

Effect of ionic strength on CoA release at different pH values solution

The effect of ionic strength on CoA release was shown in Fig. 7. Drug-loaded IPN films A3 were used in this experiment as the release matrix. Adding appropriate amounts of NaCl to the buffer solution with pH 2.1 and 7.4 produced the five different release medium. It was

Table 2 Percent weight loss of IPN CS/CM-CS films

Weight ratio of CS to CM-CS	Weight loss[a]	
	pH 2.1	pH 7.4
100:0	31.2±2.0	9.8±1.1
70:30	28.5±1.5	12.1±0.8
50:50	26.4±1.2	15.4±1.0
30:70	23.2±1.1	17.8±1.3
0:100	21.0±1.1	22.7±1.4

[a] The average value from three experiments.

found that the highest amounts of CoA released from the systems were observed at pH 2.1, and the cumulative release rate of CoA decreased with the concentration of NaCl increasing both at pH 2.1 and 7.4 values solution. This was in good agreement with the results of swelling of the IPN films shown in Fig. 3.

Effect of concentration of crosslinking agent on CoA release

The effect of crosslinking agent concentration on CoA release from the films was shown in Fig. 8. To study the effect of the crosslinking concentration on CoA release, films A3, C1–C3 were used in this study. It was found that the amount of CoA released from the films decreased with an increase of crosslinking concentration at pH 2.1 medium. It could possibly be explained by the term of degree of swelling (Table 1). The results revealed that the SR of the CoA-loaded film decreased with increasing crosslinking concentration. This was attributed to the swelling behavior of the crosslinked network. At low concentrations of crosslinking agent, the density of crosslinking was low, which made the film swell extensively. While the mesh size of the network became bigger, more CoA molecules were able to penetrate to the external environment. On the other hand, at high concentrations of crosslinking agent, the SR was limited. Therefore, the mesh size of the network was closer to the size of CoA, and CoA molecule had difficulty penetrating to the external environment. In Table 1, it was found that the degradation of the film also played an important role in the release process. The rule was that the lower the crosslinking concentration, the bigger weight loss rate, and the degradation of the films accelerated CoA release from the polymer network.

Conclusions

Biodegradable IPN complex films were successfully synthesized in this paper. The swelling behavior study of the IPN films demonstrated the pH and ionic-responsive nature of the materials. Furthermore, the films' composition, drug loading content, and crosslinking agent concentration all had relevant influence on the release property of the films. Thus, we could control the drug release rate through changing some influential factors of the drug-loaded films. These results suggested that crosslinked CS/CM-CS IPN films cloud be used as drug delivery systems.

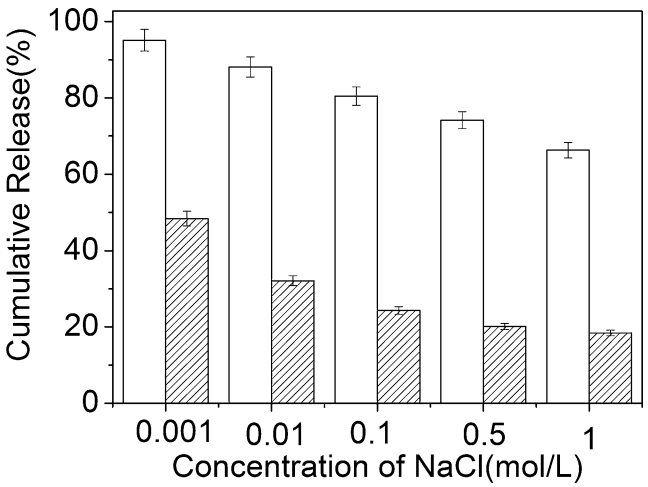

Fig. 7 Influence of ionic strength of the solution on CoA release for sample A3, pH 2.1 (*blank*), pH 7.4 (*hatched*)

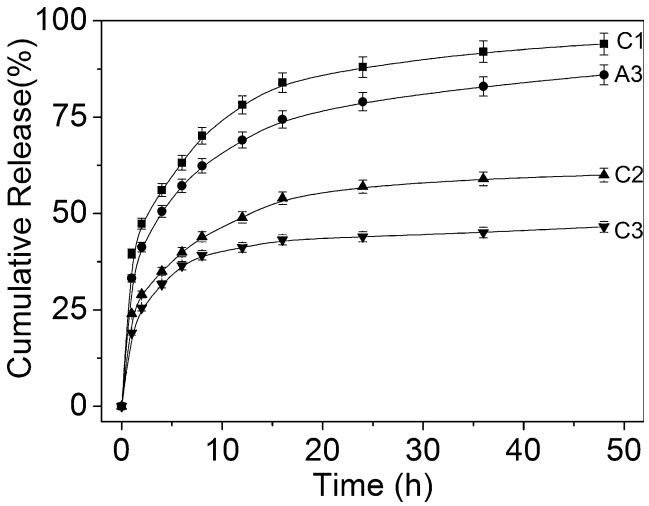

Fig. 8 Influence of crosslinking agent concentration on CoA release at pH 2.1 value solution

Acknowledgment The authors would like to thank National Natural Science Foundation of China for providing the fund (grant number, 50273010).

References

1. Wu J, Su ZG, Ma GH (2006) Int J of Pharmaceutics 315:1
2. Guo BL, Yuan JF, Yao L, Gao QY (2007) Colloid Polym Sci 285:665
3. Eve RG, Matthew S (2004) Eup J Pharm Biopharm 57:53
4. Chen LY, Tian ZG, Du YM (2004) Biomaterials 25:3725
5. Marsanoa E, Bianchia E, Vicinia S (2005) Polymer 46:1595
6. Chena SC, Wua YC (2004) J Controll Release 96:285
7. Zhao SP, Ma D, Zhang LM (2006) Macromol Biosci 6:445
8. Suzuki A, Tanaka T (1990) Nature 346:345
9. Li H, Chen J, Lam KY (2004) J Polym Sci Part B 42:1514
10. Kwon IC, Bae YH, Kim SW (1991) Nature 54:291
11. Kukufata E, Zhang YG, Janaka J (1991) Nature 351:302
12. Ren DW, Yi HF (2005) Carbohydr Research 340:2403
13. Robert L, Nicholas AP (2003) AIChE J 49:2990
14. Li GM, Cai Q, Bei JZ (2003) Polym Adva Techn 14:239
15. Yan R, Hu H, Liang F, Zong Z (1994) Chin Polym Bull 3:143
16. Dodane V, Vilivalam VP (1998) Sci Technol Today 1:246
17. Hirano S, Seino H, Akiyama I, Nonaka I (1990) In: Gebelein CG, Dunn RL (eds) Progress in biomedical polymers. Plenum, New York, p 283
18. Weiner ML (1993) In: Brine CJ, Sandford PA, Zikakis JP (eds) Advances in chitin and chitosan. Elsevier, London, p 663
19. Illum L, Davis SS (1998) Pharm Res 15:1326
20. Li J, Gong YD, Zhao NM, Zhang XF (2005) J Appl Polym Sci 98:1016
21. Maria ESM, Catiana M, Clóvis AR, Helena MW (2006) Polym Int 55:961
22. Maciel JS, Silva DA, Haroldo CB (2005) Euro Polym J l 41:2726
23. Wang Q, Du Y, Fan LH (2005) J Appl Polym Sci 96:808
24. Phyu PW, Yoshitsune SY, Hong KJ, Toshio K (2005) Polym Int 54:533
25. Muzzarelli RAA (1988) Carbohydr Polym 8:1
26. Chen SC, Wu YC, Mi FL (2004) J Control Release 3:2852
27. Thanou M, Nihot MT, Jansen M (2001) J Pharm Sci 90:38246
28. Chen LY, Du YM, Wu HQ, Xiao L (2002) J Appl Polym Sci 83:1233
29. Polk A, Amsden B, Yao KD, Peng T (1994) J Pharm Sci 83:178
30. Dumitriu S, Chornet E (1998) Adv Drug Delivery Rev 31:223
31. Yu J, Du Y, Zheng H (1999) J Wuhan Univ (Nat Sci Ed) 45:440
32. Tang R, Du Y, Zheng H, Fan LH (2001) J Wuhan Univ (Nat Sci Ed) 47:721
33. Xu J, Stephen PM, Richard AG (1996) Macromolecules 29:3436
34. Muzzarelli RAA (1986) Carbohydr Res 146:251
35. Chen LY, Du YM, Wu HQ, Xiao L (2002) J Appl Polym Sci 83:1233
36. Zhang X (1986) Handbook of practical chemistry. National Defense Industry, Beijing, p 245
37. Pramanick D, Biswas D, Ray TT, Bakr A (1994) J Polym Mater 11:41
38. Yao KD, Peng T, Xu M, Chen Y (1994) Polym Int 34:213
39. Doi M, Matsumoto M, Hirose Y (1992) Macromolecules 25:550
40. Wang Q, Du YM, Fan LH (2005) J Appli Polym Sci 96:808