Novel Physically Crosslinked Hydrogels of Carboxymethyl Chitosan and Cellulose Ethers: Structure and Controlled Drug Release Behavior

Shifeng Yan,[1] Jingbo Yin,[1] Li Tang,[1] Xuesi Chen[2]

[1]*Department of Polymer Materials, School of Materials Science and Engineering, Shanghai University, Shanghai 201800, China*
[2]*State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China*

Received 28 October 2009; accepted 18 March 2010
DOI 10.1002/app.32678
Published online 1 September 2010 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Novel hydrogels, physically crosslinked by hydrogen bonding of component polymers, were obtained by mixing aqueous solutions of carboxymethylchitosan (CMCS) with cellulose ethers including hydroxyethylcellulose (HEC) and methylcellulose (MC). The hydrogels were characterized by IR, XPS, WAXD, and SEM. The swelling and controlled drug release behaviors of hydrogels were also studied. The results indicate that intermacromolecular hydrogen bonding in CMCS/HEC is stronger than that in CMCS/MC. The swelling and drug release rate of hydrogels decrease as the interaction of component polymers increases. Both the swelling and drug release from hydrogels can be controlled by component polymer ratio. The hydrogels may be potential candidates for biomedical applications. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 119: 2350–2358, 2011

Key words: hydrogel; crosslinking; carboxymethylchitosan; hydroxyethylcellulose; methylcellulose

INTRODUCTION

Polymeric hydrogels are crosslinked, three-dimensional, hydrophilic polymer networks capable of imbibing large volumes of water without dissolving. The water retaining capacity of these materials is due to the presence of hydrophilic functional groups such as —OH, —COOH, —$CONH_2$, —CONH, —SO_3H along the polymer chains.[1] Recently, much attention has been paid to the application of polymeric hydrogels in biomedical and pharmaceutical fields, in view of their low toxicity, high biocompatibility, swelling, and mechanical properties.[2] In addition, hydrogels have the unique property of undergoing abrupt volume changes from their collapsed and swollen states in response to environmental changes, so various stimuli-sensitive hydrogels that respond to pH, temperature,[3] and other stimuli[4] have been studied experimentally and theoretically. Because of the fascinating properties of the stimuli-sensitive hydrogels, it is certain that they will have many future applications as suitable materials for the design of intelligent biomaterials and self-regulated drug delivery systems.[5]

Crosslinks of hydrogels can be established mainly through chemical method and physical method. Chemical crosslinking has some drawbacks since crosslinking agents are needed, which may be toxic and may damage incorporated drugs. Therefore, there is an increasing interest in physically crosslinked hydrogels. Several physical interactions are exploited to design hydrogels, such as ionic interactions, intermolecular reversible hydrophobic interactions, stereocomplex formation, etc.[6,7]

Polymeric hydrogels based on polysaccharides and/or cellulose have attracted much attention owing to their biocompatibilities, low toxicity, biodegradability, and comparatively low cost. Chitosan (CS), a polyaminosaccharide, exhibiting good biocompatibility, mucoadhesivity, biodegradability, low cytotoxicity, antibacterity, has been used in many fields.[8] However, the applications of CS are still limited because of its insolubility at neutral or high pH region. So, it is important to improve the soluble property of CS. Among the numerous water-soluble CS derivatives, carboxymethylchitosan (CMCS) is a

Correspondence to: J. Yin (jbyin@shu.edu.cn) or X. Chen (xschen@ciac.jl.cn).

Contract grant sponsor: National Natural Science Foundation of China; contract grant numbers: 50473061 and 50973060.
Contract grant sponsor: Shanghai Science and Technology Committee; contract grant numbers: 05JC14067 and 08JC1410300.
Contract grant sponsor: Shanghai Leading Academic Discipline Project; contract grant number: s30107.
Contract grant sponsor: Innovation Fund of Shanghai University; contract grant number: A.10-0110-07-007.

very promising candidate for some biomedical applications.[9–11] Cellulose is insoluble in water because of its high crystallinity. Cellulose ether is one kind of the most important water-soluble derivatives of cellulose. It can be grouped into two categories: ionic and nonionic. Hydroxyethylcellulose (HEC) is nonionic and compatible with a wide range of other water-soluble polymers. Methylcellulose (MC) is also nonionic. Both of them possess good biocompatibility, nonimmunogenicity, and low toxicity, etc.[12] Still, there are few studies on the CMCS/HEC and CMCS/MC hydrogels obtained by physical method.

In this study, physically crosslinked hydrogels of CMCS with cellulose ethers including HEC and MC were prepared in various compositions. The structure and swelling of hydrogels were examined. The mechanism of polymer interaction in hydrogels was discussed. The hydrogels may be potential candidates for biomedical applications such as controlled drug release system.

5-Fluorouracil (5-FU), a water-soluble fluorinated pyrimidine analog, is an antineoplastic drug, extensively used in clinical chemotherapy for the treatment of metastatic carcinomas of breast, gastrointestinal tract, pancreas, head, neck, and ovary.[13–15] 5-FU is rapidly absorbed through the blood capillaries into systemic circulation. This results in relatively low levels of drug near the site of action with the subsequent loss of efficacy and increased risk of systemic toxicity.[15,16] So, 5-FU needs long-term and sustained release in clinical application. By using controlled release formulations of 5-FU the incidence of side effects may be reduced and therapeutic effects increased.[17]

Hydrogels have been exploited for their potential as drug delivery systems. In the present study, the hydrogels of physically crosslinked hydrogels of CMCS with cellulose ethers were used to encapsulate the model drug 5-FU. The hydrogels of CMCS/HEC show a slow drug release behavior and the release rate can be controlled by formulation of CMCS and HEC.

EXPERIMENTAL

Materials

CMCS was purchased from Shanghai Seaflag Biochemistry Product Corporation (Shanghai, China), which has a viscosity of 162.5 cp for 2 wt % aqueous solution at 20°C, a deacetylation degree of 90% and a carboxymethyl group substitution degree of 80%.

HEC with the viscosity of 2 wt % aqueous solution at 20°C being 1500 cp and MC with the viscosity of 2 wt % aqueous solution at 20°C being 550 cp were purchased from Sigma-Aldrich Chemical Agent.

Fluorouracil (5-FU) was purchased from Nantong Pharmacy (Jiangsu, China) and used as model drug. The chemical structure of 5-FU is shown in Figure 10(e). Other reagents were all analytical grade.

Preparation of CMCS/cellulose ethers hydrogels

CMCS and cellulose ethers were dissolved in deionized water, respectively for the preparation of solutions (0.1 mol/L). The CMCS solution was mixed together with HEC and MC solution respectively in different molar ratios, followed by filtering with a glass filter. Hydrogels thus were obtained by casting the solutions on $4 \times 4 \times 1$ cm^3 polytetrafluoroethylene surface with subsequent drying on air at room temperature for several days until their weight unchanged. Before casting the solution, mixtures were sonicated using 200-W probe-type sonicator (JHN-M-4E, Shanghai Jump Ultrosonica Equipment, China).

Infrared spectra (IR)

The FTIR spectra of the polymeric films were recorded using a FTIR spectrophotometer (AVATAR 370, Nicolet) in the region of 4000–500 $cm^{-1.}$

X-ray photoelectron spectroscopy (XPS)

The hydrogels of CMCS/HEC (3 : 2) and CMCS/MC (1 : 1) were measured by XPS. The XPS measurements were carried out on a photoelectron spectrometer (JEOL Ltd, ESCALABMKII) using Mg Ka radiation at 12 kV and 10 mA.

Scanning electron microscopy (SEM)

The images were taken from the fracture surface of the materials. The materials were preliminary frozen in liquid nitrogen and covered by gold vapors.

Wide-angle x-ray diffractograms (WAXD)

Wide-angle X-ray diffraction patterns were analyzed using a diffactometer (D/MAX2550, Rigaku, with Cu Kα radiation at a voltage of 40 kV and 30 mA. The samples were scanned between $2\theta = 5$–$40°$ with a scanning speed of 5°/min. Prior to testing, the samples were dried and stored in a desiccator.

Measurement of the swelling ratios (SR)

To investigate the swelling properties of the hydrogels, the dried gels were incubated in buffer solutions of the designed pH (7.4) at room temperature and the swollen samples were obtained at various intervals. The swollen hydrogels were dried

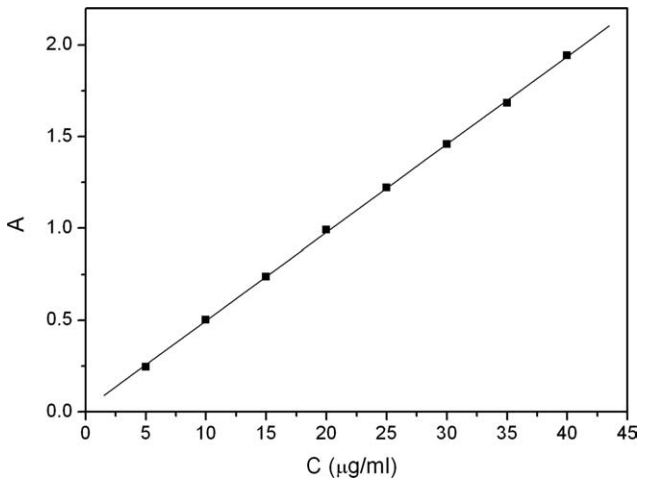

Figure 1 The standard calibration curve of the absorbance as a function of the 5-FU concentration.

superficially with filter paper, weighed and placed back in the same bath. The measurements were continued for more than 24 h. The swelling ratio was calculated using the following formula:

$$\mathrm{SR}\ (\%) = [(\mathrm{W_s} - \mathrm{W_d})/\mathrm{W_s}] \times 100$$

Where W_s and W_d are the weights of the sample in swollen and dried states, respectively.

Drug loading

The drug loaded samples were prepared by mixing the designed amount of 5-FU into the solutions of CMCS (0.1 mol/L) and HEC (0.1 mol/L) in different molar ratios. Hydrogels thus were obtained using the method mentioned above. Then the hydrogels were dried in vacuum overnight until its weight remained unchanged.

Standard absorbance curve

The standard calibration curve of the absorbance as a function of the 5-FU concentration was studied at 265 nm on the UV spectrophotometer, as shown in Figure 1. The calibration curve has a linear relationship with a correlation coefficient (r) of 0.99991, this linear relationship can be quantificationally described as the following equation:

$$A = 0.04804C - 0.01689,$$

where A is the absorbance and C the concentration (μg/mL) of 5-FU.

In vitro release at pH 7.4

The release of 5-FU from the hydrogels was determined by the dialysis method. The designed amount of drug loaded powder were carefully enveloped in the dialysis bag containing 5 mL of isotonic phosphate buffer solution (PBS; pH 7.4), ensuring the removal of the air bubbles in the dialysis bag before sealing. The samples were placed in a beaker filled with 100 mL of PBS, then were placed in a probe-type sonicator for constant sonicating. During the drug release experiment, 5 mL aliquots of the release media was taken out with reconstitution of 5 mL fresh PBS at every predetermined time interval and the concentration of the 5-FU released from this drug delivery system was monitored at 265 nm using the UV spectrophotometer.

RESULTS AND DISCUSSIONS

FTIR analysis

The IR spectra of CMCS/HEC and CMCS/MC dried hydrogels are presented in Figure 2. The absorption band at 1033 cm^{-1} in CMCS is due to C—O stretching,[18] it shifts to 1061 cm^{-1} and 1062 cm^{-1} in CMCS/HEC and CMCS/MC hydrogels, respectively. Because of the existence of C—O groups in both HEC and MC, the red shifts can not be taken as a criterion for the evaluation of intermolecular interaction. The characteristic peaks at 1588 cm^{-1} and 1410 cm^{-1} for CMCS can be attributed to asymmetric stretching and symmetric stretching vibration of carboxylic group, respectively.[19] In the spectrum of CMCS/MC (1 : 1) dried hydrogel, the positions of these two peaks almost remain unchanged, indicating the weak interaction between CMCS and MC. However, the aforementioned absorption bands in CMCS/HEC (1 : 1) dried hydrogel shift to lower wave numbers (1585 and 1407 cm^{-1}). Based upon this evidence, it can be concluded that a certain degree of interaction between CMCS and HEC molecules forms due to intermolecular hydrogen bonds.

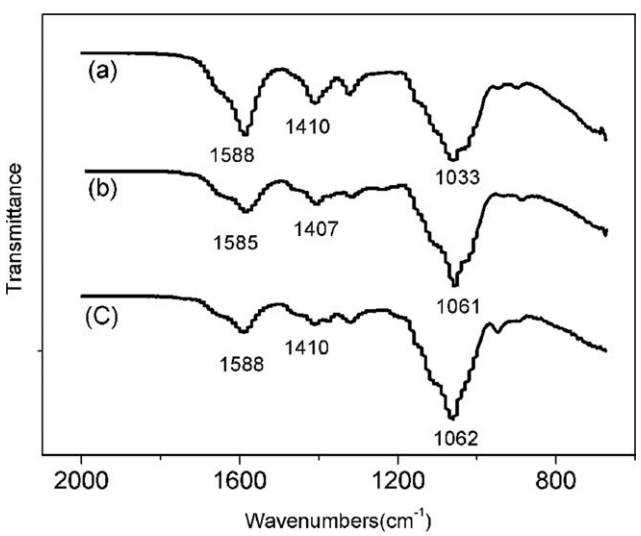

Figure 2 IR spectra of CMCS (curve a), CMCS/HEC (1 : 1) (curve b), and CMCS/MC (1 : 1) (curve c) dried hydrogels.

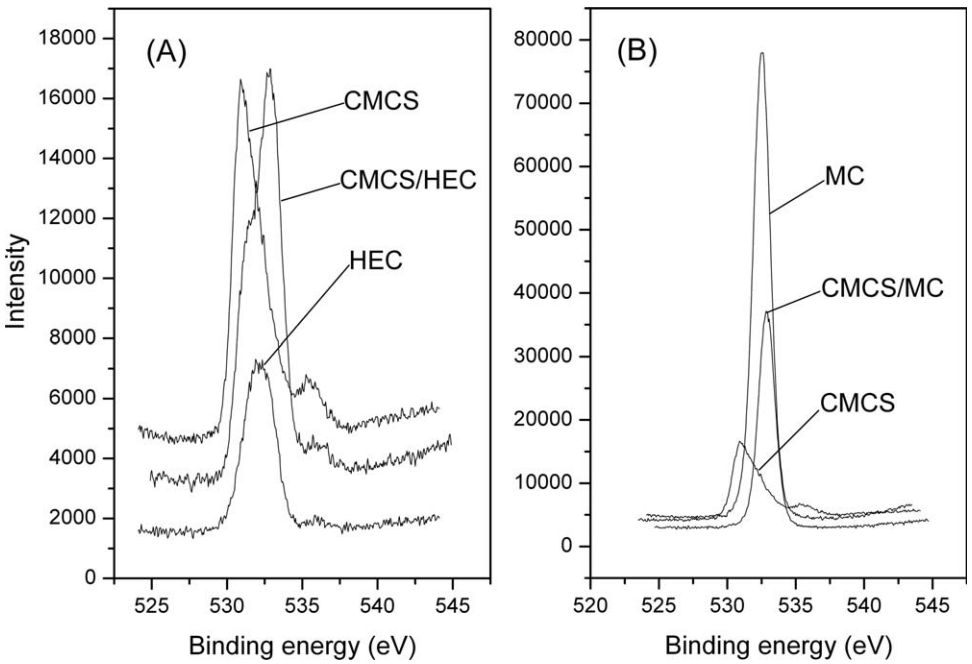

Figure 3 XPS of CMCS/HEC (1 : 1) (A) and CMCS/MC (1 : 1) (B) dried hydrogels and their component polymer films.

XPS analysis

Figure 3 shows the O_{1s} spectra of CMCS/HEC and CMCS/MC hydrogels. The O_{1s} spectra of pure CMCS, HEC and MC locate at 530.9, 532.35, and 532.6 eV, respectively. However, the O_{1s} peaks of CMCS/HEC and CMCS/MC hydrogels locate at 532.9 and 532.8 eV, respectively. Both of them shift to a high Binding Energy (BE), which results from hydrogen-bonding interactions of CMCS/HEC and CMCS/MC hydrogels.

WAXD analysis

The wide angle X-ray diffraction patterns of CMCS/HEC and CMCS/MC dried hydrogels are shown in Figure 4. The X-ray diffraction pattern of HEC [Fig. 4(A),

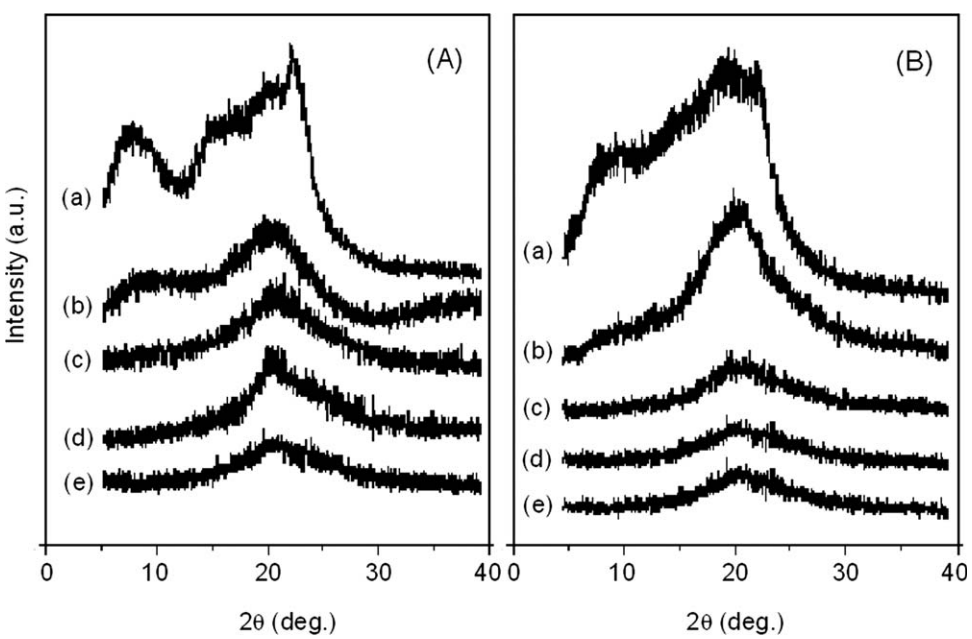

Figure 4 WAXD patterns of CMCS/HEC (A) and CMCS/MC dried hydrogels (B) with different component polymer ratios : (A, curve a) HEC; (A, curve b) CMCS : HEC = 1 : 3; (A, curve c) CMCS : HEC = 1 : 1; (A, curve d) CMCS : HEC = 3 : 1; (A, curve e) CMCS; and (B, curve a) MC; (B, curve b) CMCS : MC = 1 : 3; (B, curve c) CMCS : MC = 1 : 1; (B, curve d) CMCS : MC = 3 : 1; (B, curve e) CMCS.

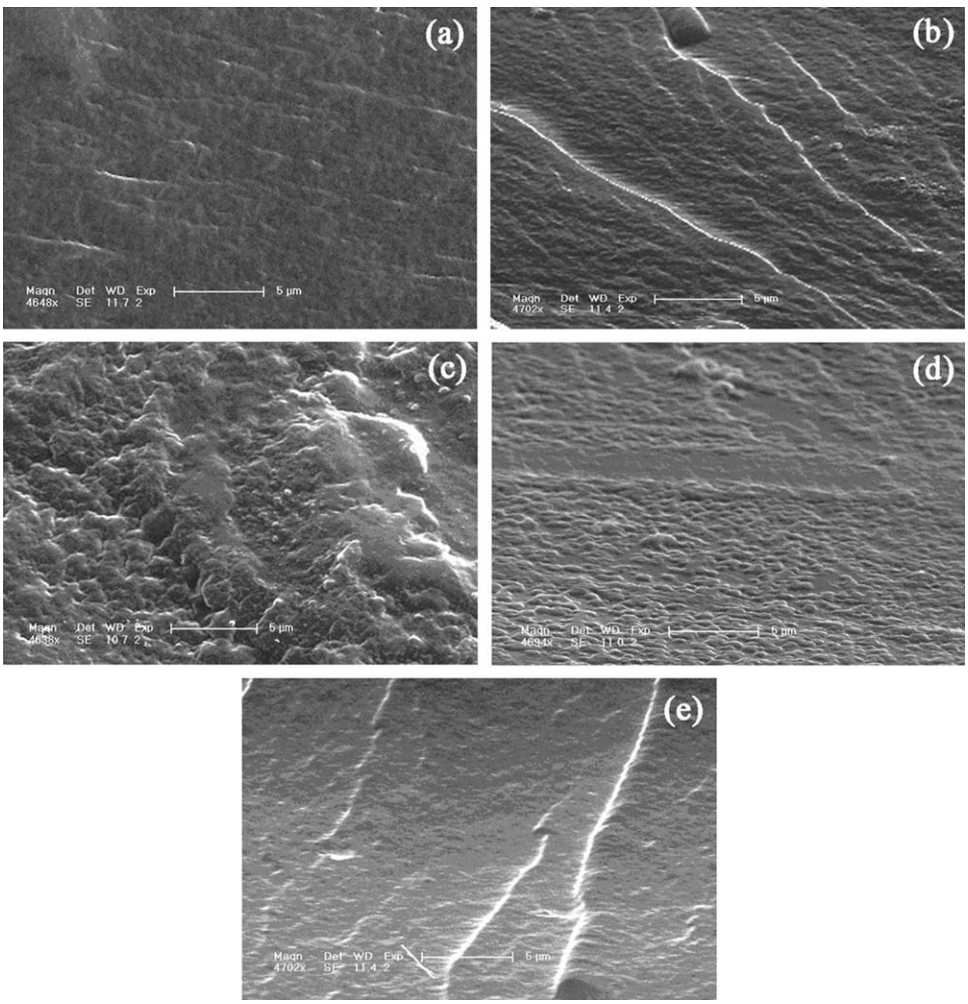

Figure 5 SEM images of CMCS/HEC dried hydrogels: (a) pure HEC; (b) pure CMCS; (c) CMCS/HEC = 3 : 1; (d) CMCS/HEC = 1 : 1; (e) CMCS/HEC = 1 : 3.

curve a] exhibits two characteristic peaks at $2\theta = 8^\circ$ and 23.2°, indicating a certain degree of crystallinity. Pure MC [Fig. 4(B), curve a] shows weak peaks at around $2\theta = 9.8^\circ$ and 22.8° due to its low crystallizability. The diffraction pattern of pure CMCS shows a broad diffraction peak centered at $2\theta = 21.4^\circ$, representing its amorphous nature. For CMCS/HEC dried hydrogels with various compositions, the X-ray diffraction patterns do not show the presence of the typical peaks for both HEC and CMCS, indicating good compatibility between HEC and CMCS. For the CMCS/MC dried hydrogels, the diffraction peak is similar to that of CMCS at the molar ratio of 3 : 1 [Fig. 4(B), curve d)] and 1 : 1 [Fig. 4(B), curve c], while the peak is similar to that of MC at the molar ratio of 1 : 3 [Fig. 4(B), curve b], indicating partial immiscibility between CMCS and MC.

SEM analysis

The miscibility of the materials was also assessed by using scanning electron microscopy to study the cross sections of the films. Figures 5 and 6 present the scanning electron micrographs of the dried films based on CMCS, HEC, and MC. It can be seen that the films of the pure CMCS, HEC, and MC have very homogeneous morphology. The CMCS/HEC dried hydrogels with various compositions are homogeneous with relatively smooth and compact cross section surfaces, especially when the molar ratio of CMCS/HEC is 1 : 3 [Fig. 5(e)]. They do not exhibit any signs of phase separation, indicating good miscibility between CMCS and HEC. In contrast, the CMCS/MC dried hydrogels have rough cross sections, confirming the interaction of these two polymers is rather weak. For CMCS/MC dried hydrogels with the component molar ratios of 1 : 1 and 1 : 3, the serious phase separation is demonstrated with a rough fracture surface and many cavities. These results indicate that CMCS and MC are not compatible, though they can form hydrogen-bonding interaction.

Figure 7 shows the morphology of films prepared by casting the blends from solutions with different pHs. For CMCS/MC dried hydrogels, it is obvious

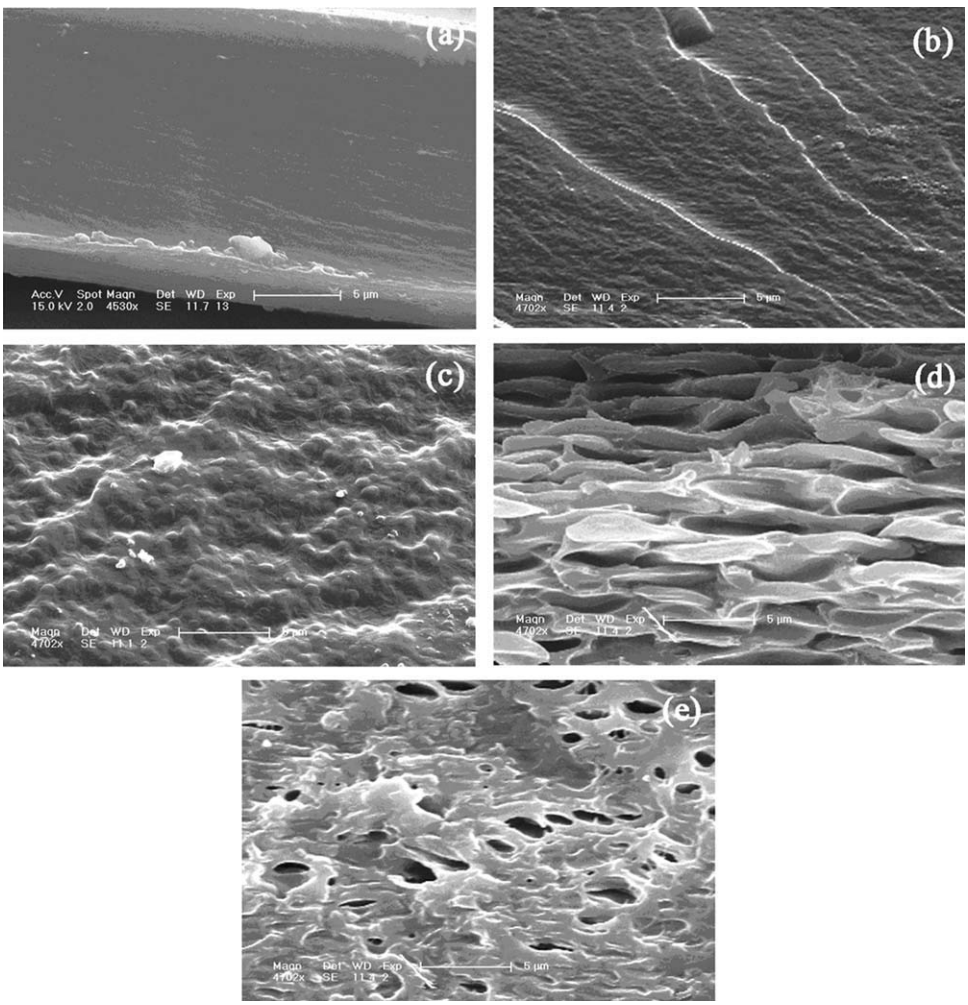

Figure 6 SEM images of CMCS/MC dried hydrogels: (a) pure MC; (b) pure CMCS; (c) CMCS/MC = 3 : 1; (d) CMCS/MC = 1 : 1; (e) CMCS/MC = 1 : 3.

that the relatively homogeneous cross section surface at pH = 2.8 changes to a rough surface with serious phase separation at pH = 8.6, indicating the transition from miscibility to immiscibility with increasing pH value. For CMCS/HEC dried hydrogels, the surface also becomes rougher with increasing pH value, but at the same pH value, the CMCS/HEC systems always show better miscibility between component polymers with more homogeneous cross section surface. The effect of pH on the cross section morphology of dried hydrogels is mainly due to the pH sensitive behavior of intermacromolecular hydrogen bonds. It is well known that intermacromolecular hydrogen bonding favor the miscibility of component polymers. The CMCS/HEC and CMCS/MC films prepared at low pH value (2.8) are miscible because of relatively strong hydrogen bonding. While at high pH, the carboxylic groups of CMCS are ionized, the intermacromolecular hydrogen drastically weakens, the blends become immiscible.

The swelling ratios of the CMCS/HEC and CMCS/MC hydrogels

The pure CMCS, HEC, and MC dried films tend to dissolve within several minutes after being placed in the buffer solutions and it is difficult to measure their swelling ratios. Figure 8 shows the optical photographs of CMCS/HEC (1 : 1) and CMCS/MC (1 : 1) hydrogels in dried and swollen states. The dried hydrogels of CMCS/HEC (1 : 1) and CMCS/MC (1 : 1) are transparent and compact. An immersion of CMCS/MC (1 : 1) dried film in buffer solutions for 18 h leads to its disintegration and partial dissociation. So, it is difficult to fetch it out from buffer solution for measurement. In comparison, the CMCS/HEC (1 : 1) hydrogel [Fig. 8(c)] expands and maintains its integrity of the structure when it is immersed in buffer solutions for 7 days. It is operational to measure the swelling ratio continually. Figure 9 depicts the swelling kinetics of CMCS/HEC hydrogels with different component polymer ratios. It can be seen that the swelling ability

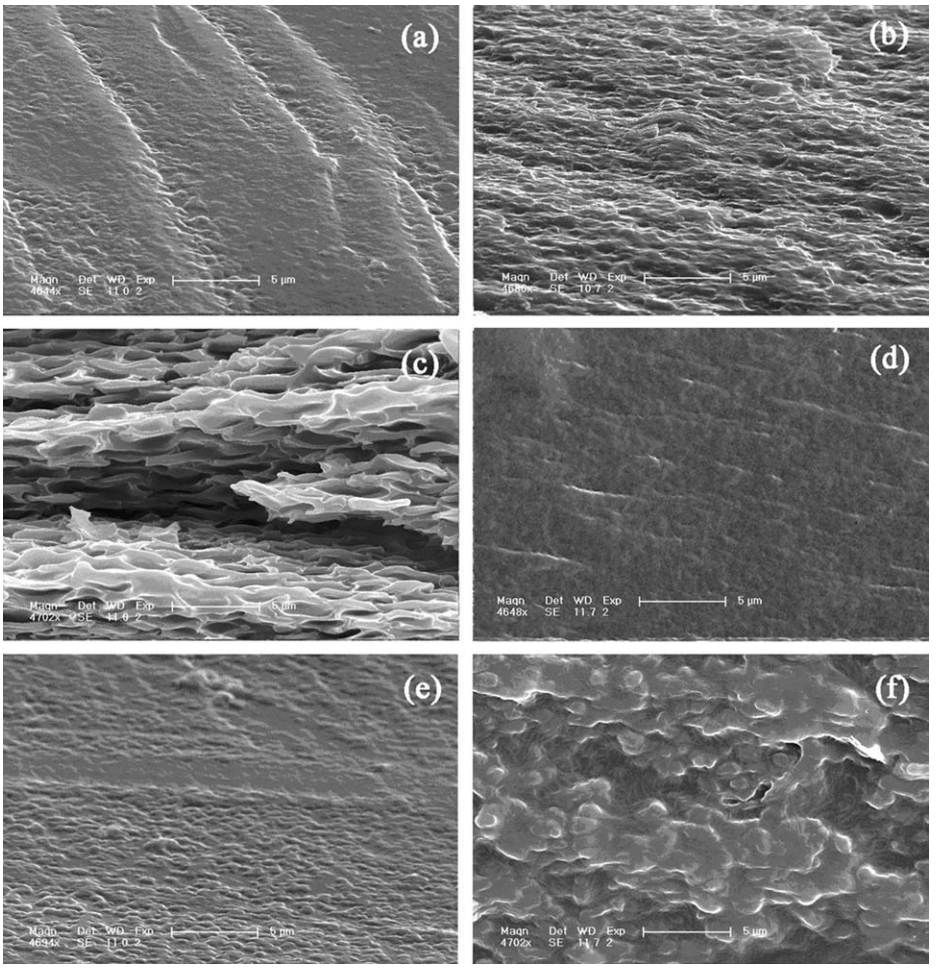

Figure 7 SEM images of CMCS/MC (1 : 1) and CMCS/HEC (1 : 1) dried hydrogels: (a) CMCS/MC, pH = 2.8; (b) CMCS/MC, pH = 7.4; (c) CMCS/MC, pH = 8.6; (d) CMCS/HEC, pH = 2.8; (e) CMCS/HEC, pH = 7.4; (f) CMCS/HEC, pH = 8.6.

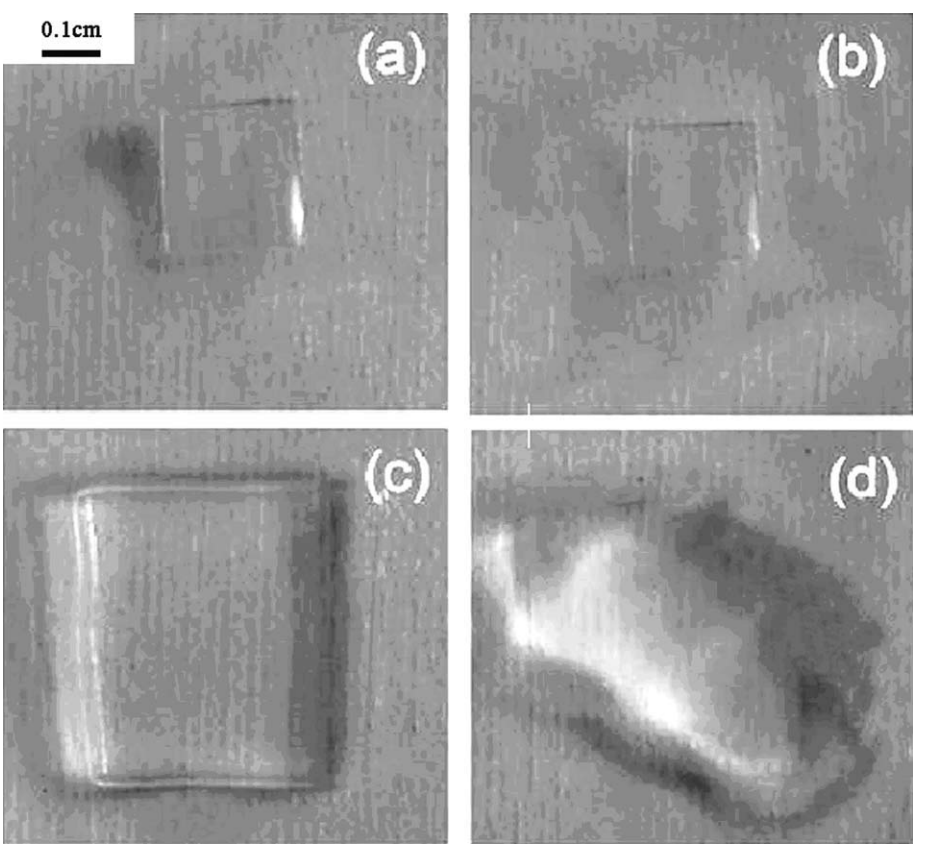

Figure 8 Optical photographs of CMCS/HEC (1 : 1) and CMCS/MC (1 : 1) hydrogels: (a) dried CMCS/HEC hydrogel; (b) dried CMCS/MC hydrogel; (c) CMCS/HEC hydrogel after 7 days; (d) CMCS/MC hydrogel after 18 h.

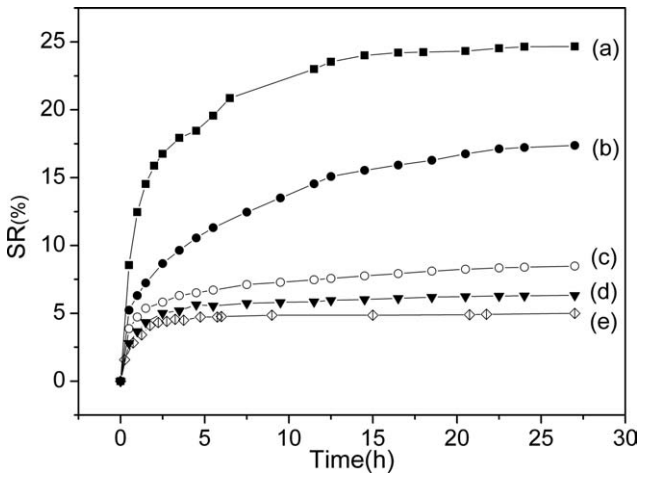

Figure 9 The relationship between swelling ratios and time in CMCS/HEC hydrogels with different component polymer ratios: (curve a) CMCS:HEC = 6 : 1; (curve b)CMCS:HEC = 3 : 1; (curve c) CMCS:HEC = 1 : 1; (curve d) CMCS:HEC = 1 : 3; and (curve e) CMCS:HEC = 1 : 6.

varies depending on the composition in the hydrogels. A highest swelling is obtained when the molar ratio of CMCS/HEC is 6:1 [Fig. 9(a)]. The swelling ratio evidently decreases with HEC content, as shown in Figure 9(b–e). In the higher CMCS:HEC ratio mixtures, hydrogen bonding can result in the formation of a physical network favoring swelling, while in the lower CMCS : HEC ratio mixtures the formation of compact complexes seems to be favored because of stronger hydrogen bonding interaction. Comparing with the results above, it can be concluded that the swelling ability of hydrogels is dependent on the interaction strength of the component polymers.

The mechanism of polymer interaction

Formation of hydrogels greatly depends on the microstructure of polymer chains, their molecular weight, and environmental conditions.[20] Formation of intermacromolecular hydrogen bonding in CMCS/HEC is easier than that in CMCS/MC, which is supported by IR, WAXD, SEM results and the swelling of hydrogels. This can be explained as due to the better flexibility of hydroxyethyl group on the HEC chains, comparing the 2, 3, and 5 position [Fig. 10(c,d)], which favors the intermacromolecular hydrogen formation in CMCS/HEC hydrogels.

In vitro drug release profile

The results above show that the pure CMCS, HEC, and MC films dissolves easily in the buffer solution, also the strength of CMCS/MC hydrogels are very poor. On the contrary, CMCS/HEC hydrogels exhibit good swelling ability and their swelling ratio

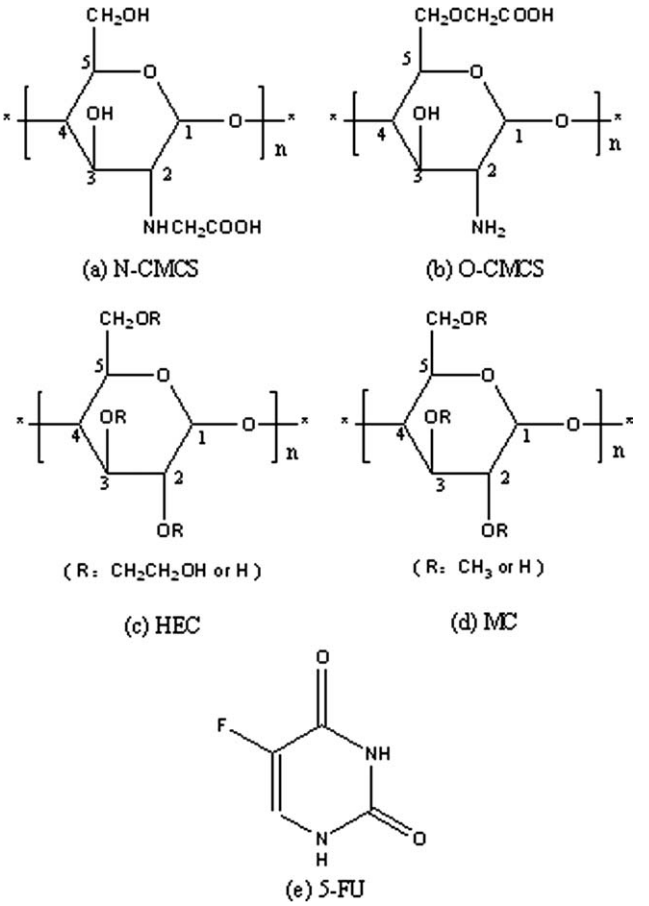

Figure 10 Chemical structures of component polymers and model drug 5-FU.

depends on the formulations of component polymers. It can be deduced that these hydrogels can be used as a novel carrier for drug delivery system. The release rate of 5-FU from the CMCS/HEC hydrogels with different formulations is described as a function of time, as shown in Figure 11. The

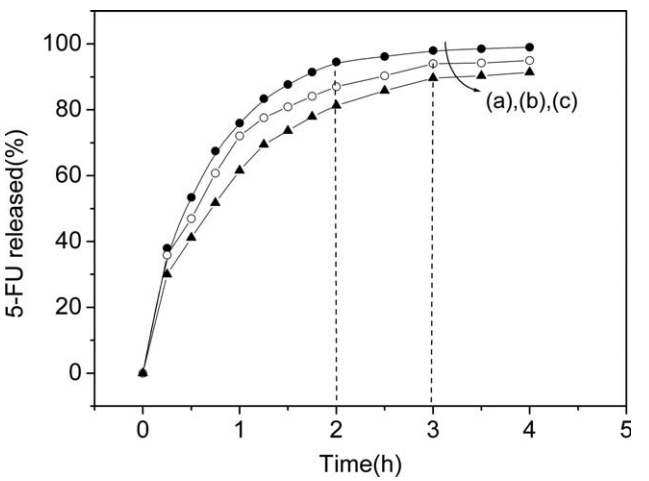

Figure 11 The release profile of 5-FU from the CMCS/HEC hydrogels: (curve a) CMCS : HEC = 3 : 1; (curve b) CMCS : HEC = 1 : 1; (curve c) CMCS : HEC = 1 : 3.

cumulative release of 5-FU reaches 94.6% within 2 h for CMCS/HEC (3 : 1) hydrogel. The release rate decreases with HEC content, as shown in Figure 11(b) and c. All the hydrogels reach an equilibrium release rate within about 3 h and then level off. The CMCS/HEC hydrogels show a slow drug release behavior compared to spray-dried hydroxyapatite-5-FU granules which deliver about 100% of incorporated drug 5-FU within only 5 min.[21] Comparing with the swelling ratio, it can be found that as the swelling ratio of the hydrogels increases, the release rate of 5-FU from the hydrogels also increases. So, both the swelling and drug release from hydrogels can be controlled by the ratio of CMCS to HEC.

CONCLUSIONS

The hydrogels of CMCS/HEC and CMCS/MC were prepared without the covalent crosslinking. Better interaction is demonstrated in CMCS/HEC hydrogels than that in CMCS/MC hydrogels, owing to stronger intermolecular hydrogen bonding in CMCS/HEC system. For the CMCS/HEC system, the interaction is excellent when the molar ratio of CMCS and HEC is 1 : 3 and the swelling behavior varies as the proportion of polymers differs. The results of controlled drug release show that as the swelling ratio of the hydrogels decreases, the release rate of 5-FU increases, indicating that the 5-FU release rate from hydrogels can be controlled by component polymer ratio.

References

1. Savas, H. L.; Olgun, G. Int J Pharm 2001, 224, 151.
2. Chena, K. S.; Yuan, A. K.; Hong, L. Mater Chem Phys 2005, 91, 484.
3. Chen, G.; Hoffman, A. S. Nature 1995, 373, 49.
4. Irie, M. Adv Polym Sci 1993, 110, 49.
5. Miyata, T.; Tadashi, U.; Nakamae, K. Adv Drug Deliver Rev 2002, 54, 79.
6. Rowley, J. A.; Madlambayan, G.; Mooney, D. J. Biomaterials 1999, 20, 45.
7. Liu, L. S.; Liu, S. Q.; Ng, S. Y.; Froix, M.; Ohno, T.; Heller, J. J Controlled Release 1997, 43, 65.
8. Yong, G. Z.; Xing, R.; Hua, Y. H. Bioorg Med Chem Lett 2005, 15, 4600.
9. Ming, X. W.; Xin, X. P. Bioorg Med Chem 2001, 11, 1699.
10. Zhu, A. P.; Chan-Park, M. B.; Dai, S.; Li, L. Colloid Surf B 2005, 43, 143.
11. Guang, C. X.; Jin, P. H. Carbohydr Polym 2003, 53, 355.
12. Arboleya, J. C.; Wilde, P. J. Food Hydrocolloid 2005, 19, 485.
13. Garcia, O.; Blanco, M. D.; Martin, J. A.; Teijon, J. M. Eur Polym J 2000, 36, 111.
14. Muzzalupo, R.; Nicoletta, F. P.; Trombino, S.; Cassano, R.; Lemma, F.; Picci, N. Colloids Surf B: Biointerfaces 2007, 58, 197.
15. Singh, B.; Chauhan, N. Acta Biomaterialia 2008, 4, 1244.
16. Hussain, M.; Beale, G.; Hughes, M.; Akhtar, S. Int J Pharm 2002, 234, 129.
17. Zhang, X. Z.; Zhuo, R. X.; Cui, J. Z.; Zhang, J. T. Int J Pharm 2002, 235, 43.
18. Zeng, M. F.; Fang, Z. P.; Xu, C. W. J Membr Sci 2004, 230, 175.
19. Fan, L. H.; Du, Y. M.; Zhang, B. Z.; Yang, J. H.; Zhou, J. P.; Kennedy, J. F. Carbohydr Polym 2006, 65, 447.
20. Nurkeeva, Z. S.; Mun, G. A.; Khutoryanskiy, V. V. Macromol Biosci 2003, 3, 283.
21. Santos, C.; Rovath, C. F.; Franke, R. P.; Almeida, M. M.; Costa, M. E. V. Ceram Int 2009, 35, 509.