

pH-sensitive interpenetrating network hydrogels based on pachyman and its carboxymethylated derivatives for oral drug delivery

Yan Hu¹ · Zhinan Mei¹ · Xianming Hu²

Received: 13 September 2014 / Accepted: 17 November 2014 / Published online: 2 May 2015
© Springer Science+Business Media Dordrecht 2014

Abstract pH-sensitive, interpenetrating polymer network hydrogels were synthesized based on pachyman and its carboxymethylated derivatives (CMP) by the confunctional crosslinker agent epichlorohydrin. The structure and morphology of pachyman/CMP (CPCS) hydrogels were characterized. In the swelling assays, the composite hydrogels maintained remarkable swelling capacity and significant good pH sensitivity. The swelling behavior was much improved by the incorporation of CMP than the pure pachyman hydrogels. While increasing the content of CMP in the hydrogels, the equilibrium swelling time was more reduced and the swelling ratio increased obviously due to the better water solubility of CMP. In the drug release study, the results indicated that CPCS hydrogels demonstrate better pH-resistant sustained drug release for bovine serum albumin, and the adjustment of the proportion of pachyman to CMP results in improved drug release behavior, which suggests that the CPCS hydrogels are promising candidates for sustained drug delivery system.

Keywords Hydrogels · Pachyman · Carboxymethyl pachyman · Drug delivery

Introduction

In recent years, the development of sustained and controlled release drug delivery systems of therapeutic agents remains a

great interest [1–3]. Among various self-regulating delivery systems, hydrogels have played an important role [4]. Hydrogels contain a highly hydrated, three-dimensional network and are known for their biocompatibility and biodegradability as well as security [5, 6], which is gaining increased attention because of their great potential in drug delivery systems, tissue engineering applications, wound healing, bioseparation, and so on [7–11]. Moreover, hydrogels with environmental sensitivity could respond to surrounding changes such as pH, temperature, ionic strength, and electric current [12–15]. In designing hydrogels for controlled drug release, pH sensitivity is an important factor in the gastrointestinal tract [16, 17]. This kind of hydrogel could not only protect the therapeutic agents from enzymatic hydrolysis or acid destruction in the upper gastrointestinal tract, but also enhance drug release at the colon by means of self-regulating to pH change [18, 19].

Trying to employ various natural polymers into drug delivery systems is a continuous subject of great interest [20–24]. Pachyman, a fungal polysaccharide, is a naturally occurring linear polysaccharide extracted from sclerotia of *Poria cocos* [25], which have gained great application in China and many other Asian countries. Many derivatives of pachyman have been prepared and developed for pharmaceutical and biomedical applications [26–28].

In our previous investigations, we fabricated two kinds of novel hydrogels based on pachyman and its derivatives (i.e. EPCS for ECH crosslinked pachyman hydrogels, and EHEP for ECH crosslinked hydroxyethyl pachyman hydrogels) by using a simple crosslinking reaction [27]. They have demonstrated pH-sensitive characteristics in response to external pH changes, especially for EHEP hydrogels. Nevertheless, EPCS hydrogels possessed unsatisfying swelling behavior in the gastrointestinal tract due to the weak solubility of pachyman as well as the limitation of its macroporous structure in hydrogel, which may lead to burst release of solutes and low

✉ Yan Hu
huyan200277@163.com

¹ College of pharmacy, South-Central University for Nationalities, Wuhan 430074, People's Republic of China

² The State Key Laboratory of Virology, College of Pharmacy, Wuhan University, Wuhan 430072, People's Republic of China

entrapment efficiency. The carboxymethylated derivative of pachyman (CMP) with good water solubility is commonly required to improve the function of pachyman. The incorporation of CMP into pachyman hydrogels may overcome the disadvantage of EPCS hydrogels in swelling and mechanical properties as well as improve the pH sensitivity due to the ionization of the COOH group in response to external pH changes [29, 30].

Moreover, multicomponent networks such as semi- or interpenetrating polymer networks (IPN) have often been developed in order to improve the mechanical properties as well as the biodegradability and the diffusion of core substance through hydrogels [31–34]. In this study, CMP was blended with pachyman to construct a matrix of an interpenetrating network as a pH-sensitive composite hydrogel for oral drug delivery. The inherent biocompatibility of pachyman and CMP makes the hydrogel materials particularly attractive for biomedical applications in drug delivery. Furthermore, bovine serum albumin (BSA) was chosen as a model protein drug to assess the potential of pachyman and CMP composite hydrogels as carriers of a pH controlled release system. The morphology of the hydrogels, the swelling characteristics and release profiles of the model drug from these carriers in simulated gastrointestinal fluids were investigated.

Experimental

Materials

Pachyman ($M_w 2.2 \times 10^5$) was extracted from the sclerotia of *Poria cocos* [35]. Epichlorohydrin and chloroacetic acid were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Bovine serum albumin (BSA) was supplied by Sigma Chemical Company (St. Louis, MO, USA). All other chemical reagents with analytical grade were obtained from commercial sources.

Preparation of carboxymethylated pachyman (CMP)

According to the method previously reported, pachyman was carboxymethylated using chloroacetic acid [26, 36, 37]. Briefly, a suspension of pachyman in a mixture of NaOH and isopropanol was stirred and dispersed in an ice bath. Then, a

mixture of chloroacetic acid dissolved in NaOH and isopropanol was slowly added under stirring. The reaction was carried out at room temperature for 3 h and then at 50 °C for 3 h. After the solution was cooled to room temperature, dilute HCl was added to neutralize the solution which was then dialyzed. Finally, CMP was lyophilized to produce the product. The sample was identified using a Fourier transform infrared spectrophotometer (FTIR, Spectrum One, Perkin Elmer, USA), and a Bruker DPX 400 MHz spectrometer, and the degree of substitution (DS) was 0.9 units of carboxymethyl group per glucose.

Preparation of pachyman/CMP (CPCS) composite hydrogels

Synthesis of pachyman/CMP composite hydrogels was carried out by an intermolecular side-chain reaction of hydroxyl groups of polymers with confunctional crosslinking agent, epichlorohydrin, in alkaline solution [38, 39]. CPCS hydrogels with pachyman-to-CMP weight ratios of 1:1, 2:1, 3:1, 4:1 were fabricated as follows. In a typical experiment, pachyman solution (5 %, w/v) and CMP solution (10 %, w/v) were prepared by dissolving 5 g pachyman or 10 g CMP in 100 mL of 2 % (w/v) NaOH at room temperature for 2 h to obtain a transparent solution. Subsequently, the two solutions were poured together as the aforementioned feed ratios and stirred for 1 h at 50 °C to get a homogeneously blended solution. After sonication, the crosslinking agent epichlorohydrin (ECH) was added to the air bubble-free pachyman-CMP solution and allowed to crosslink under constant magnetic blending at room temperature. The molar ratios of ECH to every glucose unit of polysaccharide and CMP in four different formulations were kept at 1:1. After stirring at room temperature for 30 min, the crosslinking reaction continued at 50 °C for another 3 h. Then, the hydrogels obtained were taken out and immersed in distilled water for 3 days in order to get rid of the impurities (salts and/or crosslinkers). The different feed ratios of the hydrogel samples were coded as 1CPCS, 2CPCS, 3CPCS, 4CPCS for ECH crosslinked pachyman-CMP hydrogels with compositions of 1:1, 2:1, 3:1, 4:1, respectively. Finally, the washed hydrogels were dried in a vacuum oven under room temperature and then preserved for further use. The designed formulations are listed in Table 1.

Table 1 Formulae design of CPCS hydrogels

Formulation	Weight of Pachyman g	Weight of CMP g	Volume of 2 % NaOH mL	Volume of ECH mL
1CPCS	1.0	1.0	30	0.63
2CPCS	1.2	0.6	30	0.60
3CPCS	1.2	0.4	28	0.54
4CPCS	1.6	0.4	36	0.69

Characterization of the hydrogels by FTIR

The FTIR analysis was studied to identify the chemical structure of the composite hydrogels. The four vacuum-dried samples were mixed with KBr powder and pressed into tablets under vacuum. The spectra was carried out on a Fourier transform infrared spectrophotometer (FTIR, Spectrum One, Perkin Elmer, USA) at a wavelength range of 400–4000 cm^{-1} .

Morphological study of CPCS hydrogels

Scanning electron microscopy was performed on hydrogels to gain information of their inner structure. The samples to be investigated were freeze-dried first to maintain the porous structure without any collapse. Then the hydrogels were carefully fractured to expose the interior and mounted onto the base plate and then covered with gold layer. The interior morphology of the swollen CPCS hydrogels at room temperature was obtained using a scanning electron microscope (SEM, JEOL JSM-6700 F, Japan).

Swelling measurement of CPCS hydrogels

The swelling characteristics of test hydrogels were determined by immersing dried samples to swell in aqueous solutions with different pH values in sealed containers. At appropriate time intervals, the swollen hydrogels were removed from the medium. When the excess water on the surface was wiped with filter paper, the hydrogels were weighed immediately on a sensitive balance. The media of different pH at 25 °C for the swelling studies were simulated gastric fluid (SGF, pH 1.2), simulated intestinal fluid (SIF, pH 7.4), or 0.1 M NaOH (pH 12.5).

The swelling ratios (SR) of hydrogels were calculated by using the following expression:

$$\text{SR} = (W_s - W_d) / W_d \times 100\%$$

where W_d and W_s are the weight of the dry and swollen hydrogels, respectively.

The above procedure was kept until no weight increase was observed. Constant weight was the indication that the gel had reached equilibrium swelling state. Their equilibrium water content (EWC) was calculated from the following equation:

$$\text{EWC} = (W_s - W_d) / W_d \times 100\%$$

Where W_s and W_d are the weight of swollen and dry state hydrogels, respectively.

Measurement of in vitro drug release

BSA was loaded into hydrogels by incubation, since BSA was hydrolyzed in the basic condition during the chemical preparation of hydrogels. The CPCS hydrogel disks were carefully weighed and then immersed in a 10 % (w/w) BSA solution for 3 days to ensure total swelling of the gel and maximum equilibrium of therapeutic agents. Then the swollen hydrogels with BSA were taken out and dried to a constant weight in a vacuum oven at room temperature. For the drug release test, each weighed hydrogel disk was immersed in 50 mL of dissolution medium shaken in a rotary water bath shaker at 100 rpm at 37 °C. At an appropriate interval, 3 mL of drug solution was withdrawn and immediately replaced with an equal volume of fresh buffer in order to maintain the sink condition. Using a UV spectrophotometer (UV-VIS Spectrophotometer; Lambda 35, Perkin-Elmer, Norwalk, CT, USA), the amount of

Fig. 1 FTIR spectra of **I** pachyman, **II** CMP, and **III** 1CPCS hydrogel

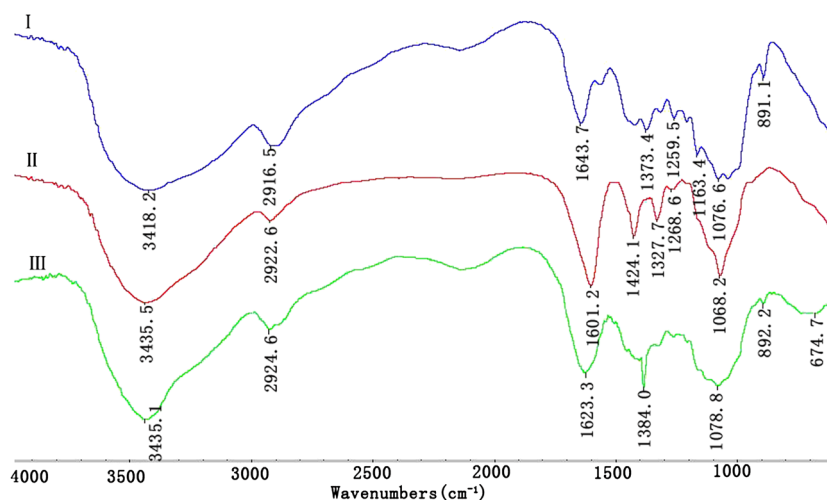
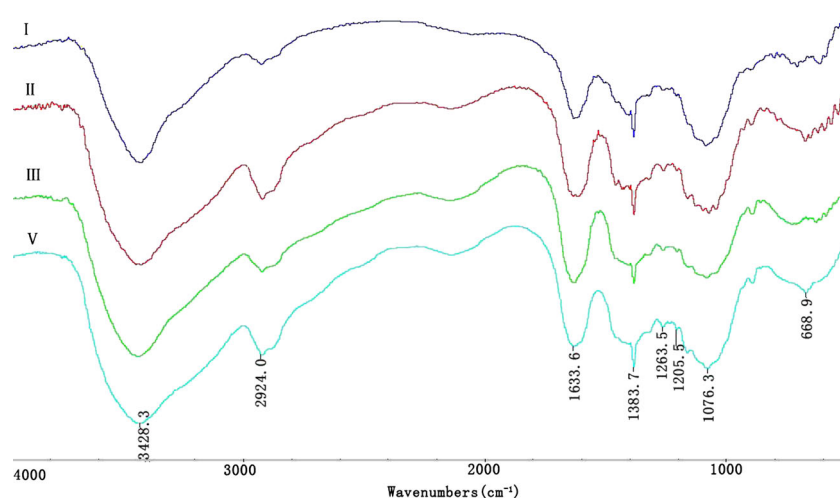


Fig. 2 FTIR spectra of **I** 1CPCS, **II** 2CPCS, **III** 3CPCS, and **IV** 4CPCS hydrogels



BSA released was determined at 280 nm. By establishing the standard calibration curves, the percentage of the cumulative amount of BSA was determined and plotted against time. The drug release of BSA was calculated from the following equation:

$$\text{Cumulative amount released (wt\%)} = M_t/M_\infty \times 100\%$$

where M_t is the weight of BSA released from the hydrogels at time t and M_∞ is the total weight loaded in the hydrogels.

Results and discussions

Hydrogel synthesis

In our previous research, we found that EPCS hydrogels exhibited weak swelling ability due to the low solubility of pachyman in the gastrointestinal tract, which could only dissolve in alkaline media [27]. In order to improve the performance of the pachyman hydrogels in aqueous solution, CMP was introduced due to its high hydrophilicity compared to pachyman. The preparation process of CMP was carried out easily using chloroacetic acid as reaction agent as reported [35].

In this work, hydrogels based on pachyman and its derivatives can be easily prepared by ECH because ECH is a convenient, base-catalyzed crosslinking agent in polysaccharide chemistry [40–42]. Once the pachyman and CMP samples were completely dissolved in the alkaline solution, this bifunctional agent could easily crosslink with the hydroxyl group in the polysaccharide molecules. In addition, the effect of crosslinking time and temperature on the extent of gelation of the CPCS hydrogel was studied. It indicated that the

crosslinking reaction maintained for 3 h at 50 °C was sufficient for complete gelation. However, before crosslinking it is necessary to remove air bubbles from the polymer solution.

Under acidic conditions, the swelling of the polymer was much slower because the carboxyl groups in CMP were in the undissociated state. However, in the neutral and alkaline environment, the repulsive interactions between the anionic groups increased due to the carboxyl groups changing into COO^- , hence the swelling of hydrogels in this environment increased. According to this mechanism, four formulations based on different proportions of pachyman to CMP were employed in order to get the optimal feed ratio for drug delivery research.

IR characterization

FTIR is an effective tool for confirming the chemical reaction in the polymer structure. Figure 1 presents the FTIR spectra of pachyman, CMP, and the hydrogel product CPCS based on ECH crosslinking. All the FTIR spectra exhibited broad peaks around 3,000–4,000 cm^{-1} corresponding to O-H stretching vibration modes. In the IR spectra of CPCS, the peak at 2,800–3,000 cm^{-1} signed to C-H stretching and 1,158 cm^{-1} corresponding to the C-O-C stretching displayed an obviously increase in their intensity compared with the spectra of

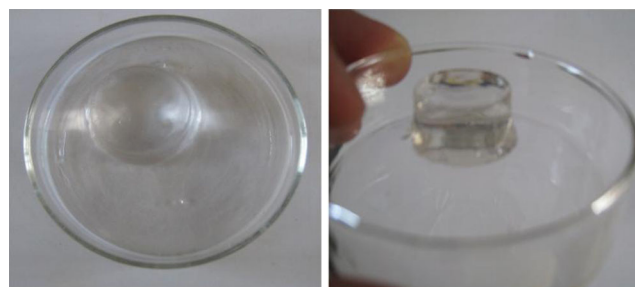


Fig. 3 Macroscopic photos of 1CPCS hydrogels

pachyman and CMP, which indicated that the crosslinking reaction occurred between the hydroxyl groups of the glucose unit and crosslinking agent ECH.

The FTIR spectra of CPCS hydrogels with different pachyman and CMP composition ratios were shown in Fig. 2. Compared with four spectra of CPCS, the peaks at 890.04 cm^{-1} assigned to the characteristic absorption peak of β pyranose gradually increased with the increasing ratio of pachyman in CPCS and the strong peaks at $1,620\text{--}1,580\text{ cm}^{-1}$ moved to high wave number owing to the crosslinking reaction. Additionally, in the four spectra, the strength of the peaks at $1,200\text{--}1,000\text{ cm}^{-1}$ assigned to the characteristic absorption peak of the

C-O-C stretching did not significantly change due to the same crosslinking ratio in four hydrogels.

Morphological studies

The hydrogels based on pachyman and CMP were fully transparent in appearance (Fig. 3). SEM images with different magnification were obtained to characterize the microstructure of the freeze-dried 1CPCS, 3CPCS, and 4CPCS gels, and are presented in Fig. 4. According to cross-sectional SEM images, it should be highlighted that these hydrogels had a continuously regular and porous structure which was

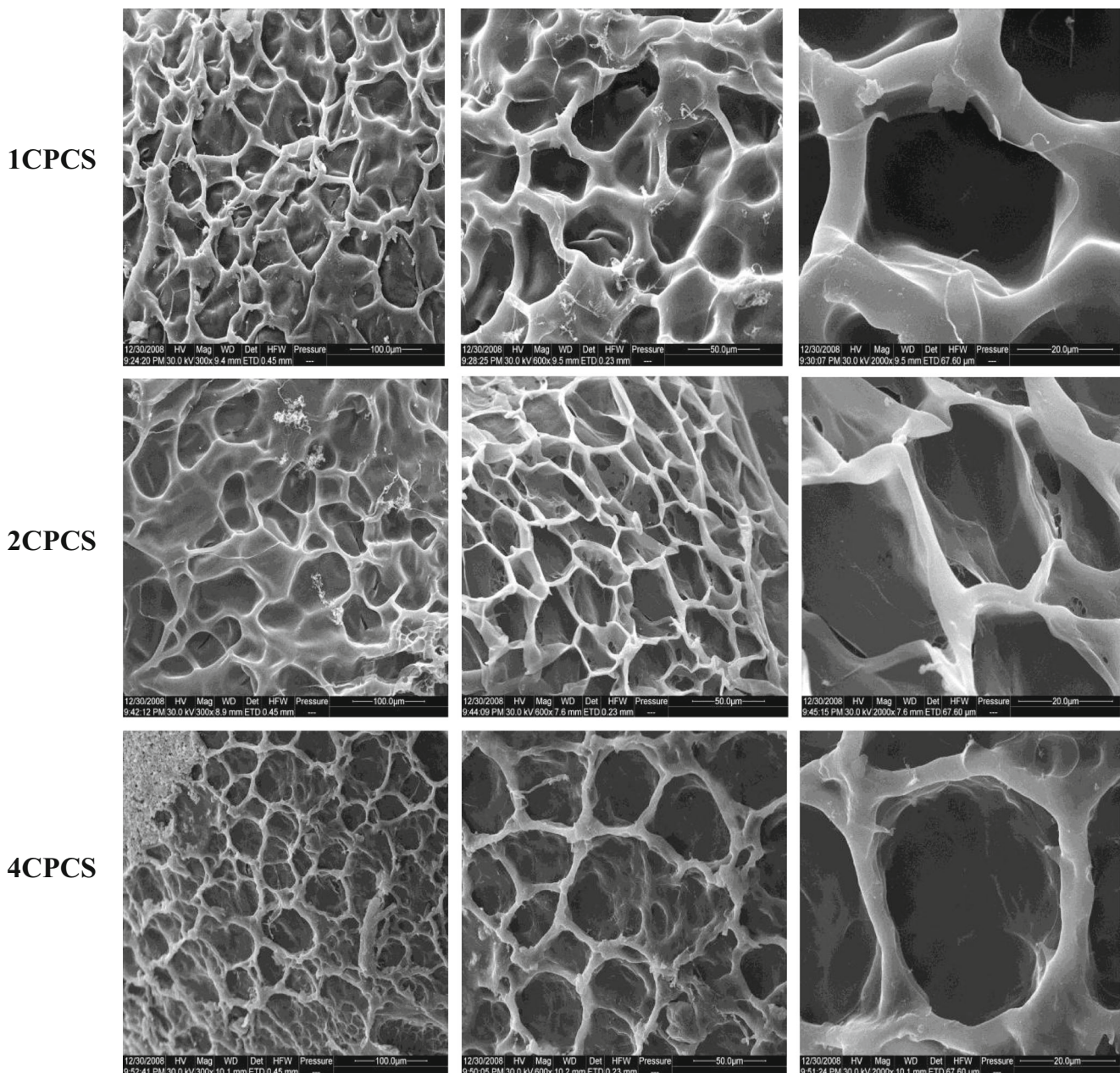


Fig. 4 SEM images of 1CPCS hydrogels with different magnification

the result of crystal formation in the freeze-drying step. As shown in the figures, the pores with diameters in the range of 30–40 μm appeared regularly and obviously in the hydrogels, which facilitated the diffusion of the solutes into the hydrogel network. The three-dimensional interconnection between pores confirmed the network formed by cross-linking with epichlorohydrin.

In addition, comparing the three composite hydrogels in the figures, there were some differences in microstructure. Because of the large content of CMP, the skeletal structure of 1CPCS hydrogels was loose, and the gap between the pores was larger and more collapsed. While in the 3CPCS and 4CPCS hydrogels, due to the large content of pachyman, the three-dimensional structure with smaller holes seemed more solid, and the connecting parts between the holes were more rigid, which may be the reason why the 4CPCS hydrogel swells slower than other samples.

Effect of pH on the swelling properties of the hydrogels

The swelling profile has a great relationship with the diffusion and release behavior of drugs through the gels. In order to gain insight into the solution and solutes transport process through CPCS hydrogels, the effects of pH values of the swelling medium and feed ratio of pachyman to CMP on the swelling behavior of hydrogels were studied.

Swelling behaviors of the CPCS hydrogels with different feed ratio were studied in SGF, SIF, and in alkaline media, respectively, as plotted in Fig. 5. The same swelling procedures were kept for all samples. As seen here, these hydrogels swelled rapidly in water during the first few hours and then gradually steadied up to the equilibrium swelling in approximately 10 h or more. Moreover, as shown in Fig. 5, the swelling behavior of the CPCS in different pH buffers exhibited obviously

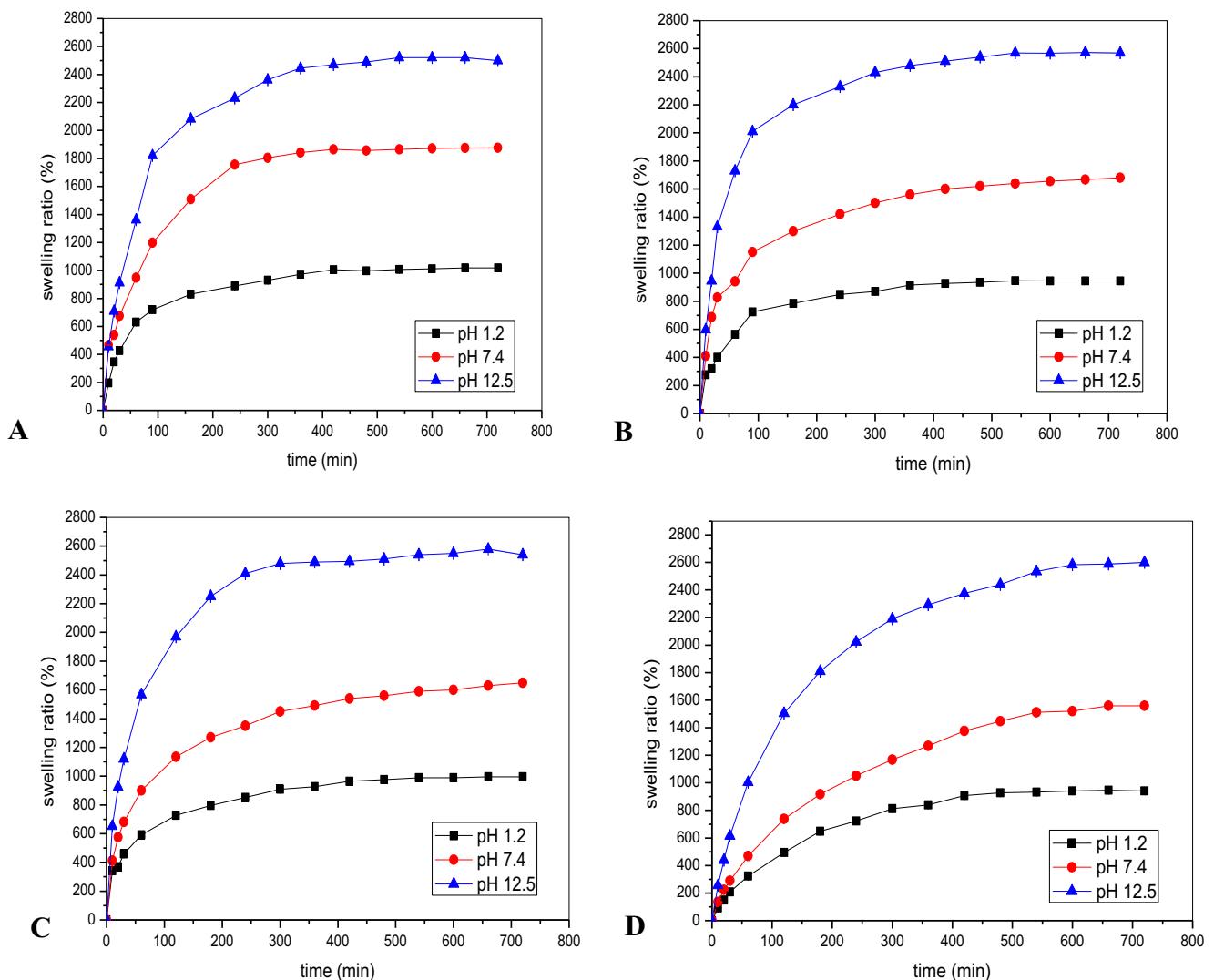


Fig. 5 Swelling behaviour of four hydrogels at different pHs at 25 °C. (a:1CPCS; b:2CPCS; c:3CPCS; d:4CPCS)

pH sensitivity. It was noted that the SR degree increased with the help of increasing pH, especially in basic medium. A probable explanation is that on the basis of the good solubility of pachyman in basic solution, the CPCS hydrogels also demonstrated better swelling behavior in basic medium. Therefore, in pH 12.5, all the CPCS hydrogels exhibited their biggest swelling ratios than for other pH values.

Compared with these four hydrogels, it can be found that the content of CMP also has certain influence on the pH sensitivity. In the 1CPCS hydrogel, due to the larger content of CMP, the equilibrium swelling ratio under neutral conditions was 1.8 times more than the swelling in the acidic condition. With the decrease of CMP content, the difference of swelling behavior in neutral and in acidic conditions gradually reduced, but the difference between neutral and alkaline conditions increased. For example, for 4CPCS hydrogels under alkaline conditions, the equilibrium swelling ratio was 1.7 times more than that under neutral conditions. But for the 1CPCS hydrogel, the swelling ratio in alkaline conditions was only 1.3 times more than that under neutral conditions.

In addition, in the different pH environments, the CPCS hydrogels exhibited significantly different swelling profiles. The swelling ratio for each gel was the biggest in alkaline conditions while it was second-largest in neutral conditions. The minimum rate of swelling was observed in acidic medium. These observations indicate a good pH-sensitive swelling behavior.

Effect of feed ratio of pachyman to CMP on the swelling properties of the gel

As shown in Fig. 6a, the equilibrium swelling ratio of four hydrogels did all not exceed 1,000 and all the CPCS hydrogels achieved their swelling equilibrium after 10 h of the test, which was much shorter than 50 h for the pure pachyman hydrogels. Compared with the swelling behavior of four hydrogels in acidic medium, it was noted that the swelling ratio decreased accompanied by the increasing pachyman ratio. Because of the smaller content of CMP in 4CPCS, the hydrogels yielded less swelling properties than the other three hydrogels in acidic medium. However, 1CPCS with more CMP content swelled slightly larger than the other three due to the better solubility and hydrophilicity of the polymer network. Therefore, the lowest EWC value of ~ 913 was for 4CPCS hydrogels.

Figure 6b shows the swelling curves of four hydrogels in neutral conditions. In this buffer, it was found that the swelling tendencies of these four hydrogels were obviously different. With increasing pachyman content, the swelling ratio of the hybrid hydrogels decreased. Therefore, in the figure, the

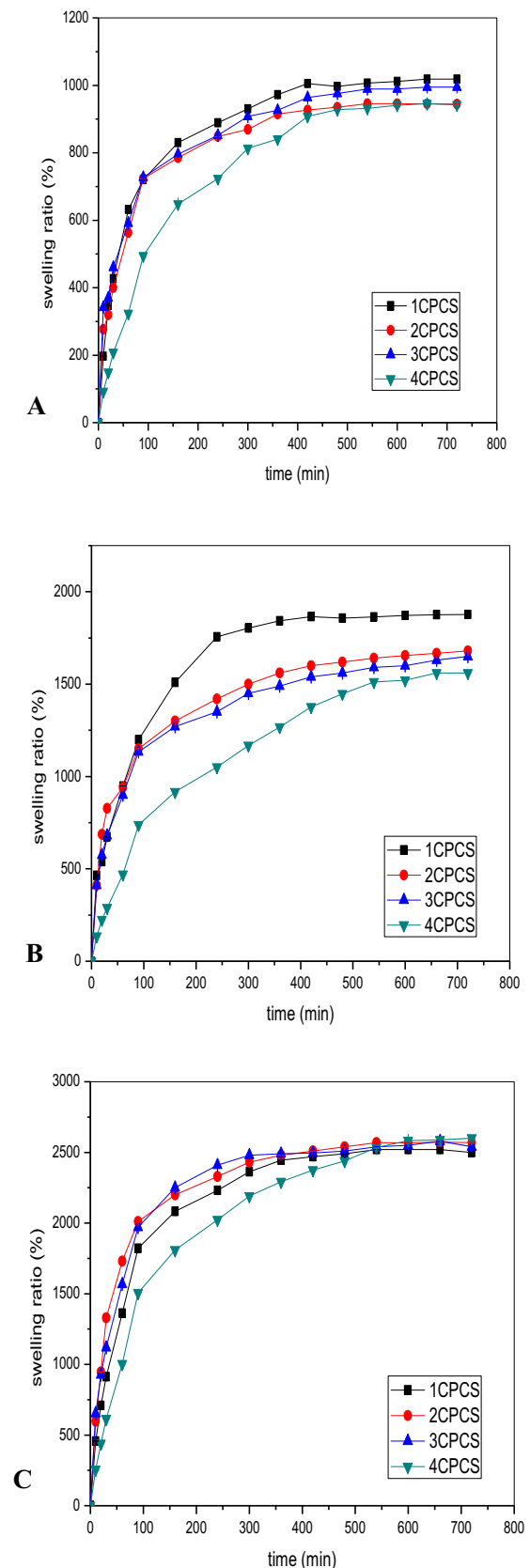


Fig. 6 Swelling behaviour of four hydrogels at different pHs at 25 °C. (a, pH 1.2; b, pH 7.4; c, pH 12.5)

swelling ratios are in the following order: 1CPCS>2CPCS>3CPCS>4CPCS. The results strongly demonstrated that CMP contributed to the swelling of the CPCS hydrogels. Because of the large content of CMP, the equilibrium swelling ratio of 1CPCS can reach up to 1,900.

Figure 6c is a swelling curve of four hydrogels in alkaline medium. It was found that the swelling performances of the four hydrogels were very close to each other in this medium. Their equilibrium swelling ratio was around 2,500. Moreover, the content of CMP did not greatly affect the swelling behaviors of these hydrogels.

Figure 7 shows the two-step swelling behaviors of four hydrogels. In the first 2 h, all the hydrogels swelled slowly. But the biggest degree of swelling appeared in 1CPCS. The swelling ratios for four hydrogels all enhanced drastically with the increasing pH values. In neutral conditions, the EWC value of ~1,700 was for 1CPCS hydrogels, and 1,500 for both 2CPCS and 3CPCS. The smallest value was for 4CPCS due to the largest content of pachyman in the hydrogels, which displayed similar swelling rates in the two distinct swelling mediums.

Drug incorporation and in vitro release

In order to investigate the drug release behavior in the gastrointestinal tract, the hydrogels with different feed ratio were immersed in the simulated gastric conditions (SGF) and in the intestinal conditions (SIF) by only changing the dissolution medium.

Figure 8 shows the BSA release profiles from hydrogels with different feed ratios. Figure 8 a and b demonstrate the

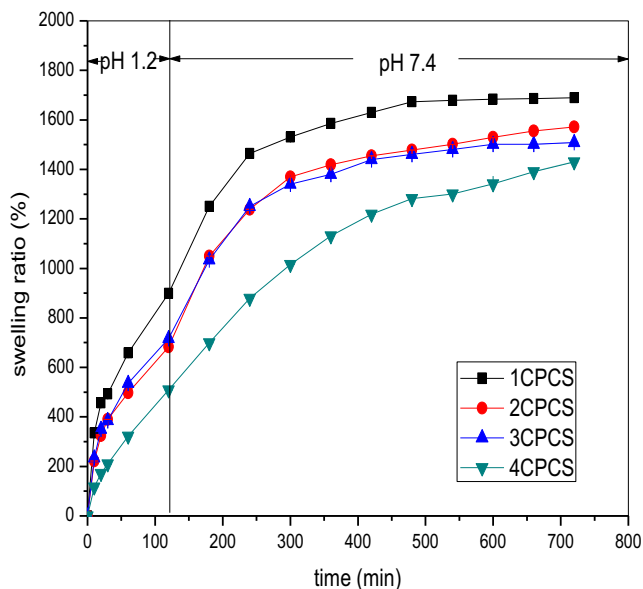


Fig. 7 Swelling characteristics of CPCS hydrogels by immersing dried test samples to swell in a solution at pH 1.2 for 2 h and subsequently in another solution at pH 7.4 at 37 °C

cumulative release profiles of 2CPCS in SIF simply and in simulated oral administration from the stomach into the intestines within 10 h, respectively. The maximum release amount in SIF was about 88 %, as seen in the chart. But in stimulated gastrointestinal tract conditions, the maximum release amount was approximately 79 %, which was less than that in the pH 7.4 medium. The results revealed that the release medium was responsible for the different release profiles of BSA at different time points. A faster release in SIF was due to the good swelling of CPCS hydrogels in neutral conditions.

The cumulative release profiles of 3CPCS in SIF only and simulated oral administration from the stomach into the intestines are shown in Fig. 8. The maximum release amount in SIF maintained at 70 %, as seen in the chart. But in stimulated gastrointestinal tract conditions, the maximum release amount was nearly 59 %, which was less than that in SIF only. In comparison with 2CPCS, a faster release in SIF was also seen in 3CPCS. Because of the pH sensitivity of CPCS hydrogels,

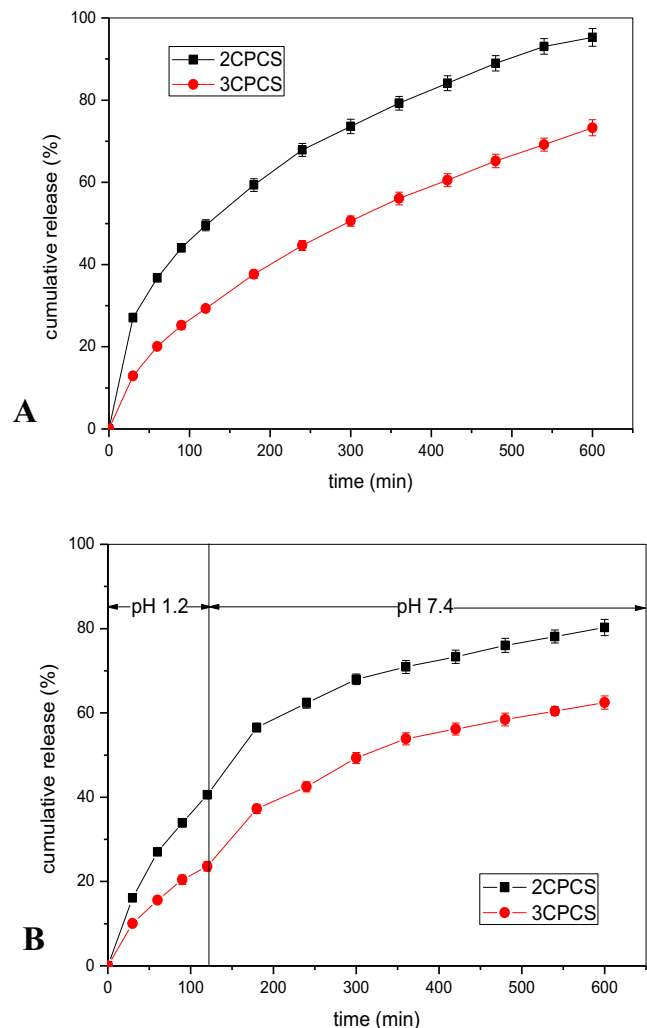


Fig. 8 BSA release profile of 2CPCS and 3CPCS hydrogels at different pHs. (a, pH 7.4; b, pH 1.2~7.4)

the drug release in the neutral was higher than in the acidic conditions.

Figure 8b shows the cumulative release profiles of BSA from SGF to SIF in 10 h. From the profiles, it can be seen that both two investigated hydrogels showed the burst release of drugs to some extent in SGF in the first 2 h. The cumulative release amount of BSA from 2CPCS and 3CPCS were about 79 and 59 %, respectively. Compared to 3CPCS, the drug release from 2CPCS was slightly higher. The main reason for this behavior was because 2CPCS with higher content of CMP swelled much greater than 3CPCS.

As shown in the profiles, it can be seen that both the 2CPCS and 3CPCS exhibited sustained and controlled release for BSA. However, for the 3CPCS hydrogel, the burst release of the protein drug was depressed much more than 2CPCS in the harsh acidic environment. In addition, a statistically significant release in 3CPCS was observed in SIF than in SGF solutions. Therefore, the results suggest that the 3CPCS with the typical pH sensitive characteristic will be a good candidate for site-specific protein drug delivery in the intestine.

Conclusions

pH-sensitive interpenetrating network hydrogels based on pachyman and its derivatives (CMP) were successfully prepared by chemical crosslinking. These hydrogels displayed remarkable swelling capacity and stability in a wide range of pH values by the incorporation of CMP compared to pure pachyman hydrogels. In vitro release profiles showed that the release of BSA in SIF (pH 7.4) was much higher compared to SGF (pH 1.2) and decreased with increasing pachyman content. Therefore, CPCS hydrogels fabricated in this work is an interesting and pH-sensitive device for a sustained and controlled release drug delivery systems. Moreover, this research opens up another interesting perspective space for pachyman developing, which may have great potential application in the pharmaceutical or biochemical fields.

Acknowledgments The authors are grateful to the financial support from the National Natural Science Foundation of China (No.81401510) and the Fundamental Research Funds for the Central Universities, South-Central University for Nationalities (CZQ12019).

References

- Qiu Y, Park K (2012) Environment-sensitive hydrogels for drug delivery. *Adv Drug Deliv Rev* 64:49–60
- Matricardi P, Meo CD, Coviello T, Hennink WE, Alhaique F (2013) Interpenetrating polymer networks polysaccharide hydrogels for drug delivery and tissue engineering. *Adv Drug Deliv Rev* 65:1172–1187
- Vashist A, Gupta YK, Ahmad S (2012) Interpenetrating biopolymer network based hydrogels for an effective drug delivery system. *Carbohydr Polym* 87:1433–1439
- Singh B, Bala R (2014) Polysaccharide based hydrogels as controlled drug delivery system for GIT cancer. *Int J Biol Macromol* 65:524–533
- Hoffman AS (2012) Hydrogels for biomedical applications. *Adv Drug Deliv Rev* 64:18–23
- Nguyen MK, Alsberg E (2014) Bioactive factor delivery strategies from engineered polymer hydrogels for therapeutic medicine. *Prog Polym Sci* 39:1235–1265
- Hunt JA, Chen R, Veen TV, Bryan N (2014) Hydrogels for tissue engineering and regenerative medicine. *J Mater Chem B* 2:5319–5338
- Li P, Dou XQ, Feng CL, Zhang D (2013) Mechanical reinforcement of C2-phenyl-derived hydrogels for controlled cell adhesion. *Soft Matter* 9:3750–3757
- Wang H, Han A, Cai Y, Xie Y, Zhou H, Long J, Yang Z (2013) Multifunctional biohybrid hydrogels for cell culture and controlled drug release. *Chem Commun* 49:7448–7450
- Silva R, Singh R, Sarker B, Papageorgiou D, Juhasz JA, Roether JA, Cicha L, Kaschta J, Schubert DW, Chrissafis K, Detscha R, Boccaccini AR (2014) Hybrid hydrogels based on keratin and alginate for tissue engineering. *J Mater Chem B* 2:5441–5451
- Patenaude M, Smeets NB, Hoare T (2014) Designing injectable, covalently cross-linked hydrogels for biomedical applications. *Macromol Rapid Commun* 35:598–617
- Pasale SK, Cerroni B, Ghugare SV, Paradossi G (2014) Multiresponsive hyaluronan-p(NiPAAm) “Click”-linked hydrogels. *Macromol Biosci* 14:1025–1038
- Bocourt M, Bada N, Acosta N, Bucioc E, Penichea C (2014) Synthesis and characterization of novel pH-sensitive chitosan-poly(acrylamide-coitaconic acid) hydrogels. *Polym Int* 63:1715–1723
- Heilmann S, Kuchler S, Wischke C, Lendlein A, Steind C, Schäfer-Korting M (2013) A thermosensitive morphine-containing hydrogel for the treatment of large-scale skin wounds. *Int J Pharm* 444: 96–102
- Hamcerencu M, Desbrieres J, Khoukh A, Popaa M, Riess G (2011) Thermodynamic investigation of thermoresponsive xanthan-poly (N-isopropylacrylamide) hydrogels. *Polym Int* 60:1527–1534
- Yang J, Chen J, Pan D, Wan Y, Wang Z (2013) pH-sensitive interpenetrating network hydrogels based on chitosan derivatives and alginate for oral drug delivery. *Carbohydr Polym* 92:719–725
- Gupta P, Vermani K, Garg S (2002) Hydrogels: from controlled release to pH-responsive drug delivery. *Drug Discov Today* 7: 569–579
- Chaturvedi K, Ganguly K, Nadagouda MN, Aminabhavi TM (2013) Polymeric hydrogels for oral insulin delivery. *J Control Release* 165:129–138
- Koetting MC, Peppas NA (2014) pH-Responsive poly(itaconic acid-co-N-vinylpyrrolidone) hydrogels with reduced ionic strength loading solutions offer improved oral delivery potential for high isoelectric point-exhibiting therapeutic proteins. *Int J Pharm* 471:83–91
- Widjaja LK, Bora M, Chan PNP, Lipik V, Wong TTL, Venkatraman SS (2013) Evaluation of the in vitro and in vivo biocompatibility of carrageenan-based hydrogels. *J Biomed Mater Res Part A* 00A:1–10
- Boddhi S, Kipper MJ (2010) Engineering nanoassemblies of polysaccharides. *Adv Mater* 22:2998–3016
- Kulterer MR, Reichel VE, Kargl R, Köstler S, Sarbova V, Heinze T, Stana-Kleinschek K, Ribitsch V (2012) Functional polysaccharide composite nanoparticles from cellulose acetate and potential applications. *Adv Funct Mater* 22:1749–1758

23. Shelke NB, James R, Laurencin CT, Kumbar SG (2014) Polysaccharide biomaterials for drug delivery and regenerative engineering. *Polym Adv Technol* 25:448–460
24. Klak MC, Lefebvre E, Remy L, Agniel R, Picard J, Giraudier S, Larreta-Garde V (2013) Gelatin-alginate gels and their enzymatic modifications: controlling the delivery of small molecules. *Macromol Biosci* 13:687–695
25. Chihara G, Hamuro J, Maeda Y, Arai Y, Fukuoka F (1970) Antitumour polysaccharide derived chemically from natural glucan (pachyman). *Nature* 225:943–944
26. Hu Y, He XR, Lei L, Liang SC, Qiu GF, Hu XM (2008) Preparation and characterization of self-assembled nanoparticles of the novel carboxymethyl pachyman-deoxycholic acid conjugates. *Carbohydr Polym* 74:220–227
27. Hu Y, Zhou X, Lu Y, Hu C, Hu XM (2009) Novel biodegradable hydrogels based on pachyman and its derivatives for drug delivery. *Int J Pharm* 371:89–98
28. Hu Y, Yang T, Hu X (2012) Novel polysaccharides-based Nanoparticle carriers prepared by polyelectrolyte complexation for protein drug delivery. *Polym Bull* 68:1183–1199
29. Dulong V, Cerf DL, Picton L, Muller G (2006) Carboxymethylpullulan hydrogels with a ionic and/or amphiphilic behavior: swelling properties and entrapment of cationic and/or hydrophobic molecules. *Coll Surf A* 274:163–169
30. Xiao Y, Xu W, Zhu Q, Yan B, Yang D, Yang J, He X, Liang S, Hu X (2009) Preparation and characterization of a novel pachyman-based pharmaceutical aid. II: a pH-sensitive, biodegradable and biocompatible hydrogel for controlled release of protein drugs. *Carbohydr Polym* 77:612–620
31. Liang S, Liu L, Huang Q, Yam KL (2009) Preparation of single or double network chitosan/poly(vinyl alcohol) gel films through selective cross-linking method. *Carbohydr Polym* 77:718–724
32. Hoare TR, Kohane DS (2008) Hydrogels in drug delivery: progress and challenges. *Polymer* 49:1993–2007
33. Zhao S, Ma D, Zhang L (2006) New semi-interpenetrating network hydrogels: Synthesis, characterization and properties. *Macromol Biosci* 6:445–451
34. Cui L, Jf J, Guo Y, Liu Y, Zhu P (2014) Preparation and characterization of IPN hydrogels composed of chitosan and gelatin cross-linked by genipin. *Carbohydr Polym* 99:31–38
35. Wang YF, Zhang LN, Ruan D (2004) Preparation and structure of five derivatives of β -(1–3)-D-glucan isolated from poria cocos sclerotium. *Chin J Polym Sci* 22:137–145
36. Wang Y, Mo Q, Li Z, Lai H, Lou J, Liu S, Mao J (2012) Effects of degree of carboxymethylation on physicochemical and biological properties of pachyman. *Int J Biol Macromol* 51:1052–1056
37. Wang YF, Zhang LN (2006) Chain conformation of carboxymethylated derivatives of (1–3)- β -d-glucan from Poria cocos sclerotium. *Carbohydr Polym* 65:504–509
38. Zhou J, Chang C, Zhang R, Zhang L (2007) Hydrogels prepared from unsubstituted cellulose in NaOH/Urea aqueous solution. *Macromol Biosci* 7:804–809
39. Liu YY, Fan XD (2003) Preparation and characterization of a novel responsive hydrogel with a cyclodextrin-based macromonomer. *J Appl Polym Sci* 89:361–367
40. Chang CY, Duan B, Cai J, Zhang L (2010) Superabsorbent hydrogels based on cellulose for smart swelling and controllable delivery. *Eur Polym J* 46:92–100
41. Denizli BK, Can HK, Rzaev ZMO, Guner A (2004) Preparation conditions and swelling equilibria of dextran hydrogels prepared by some crosslinking agents. *Polymer* 45:6431–6435
42. Zhang LM, Wu CX, Huang JY, Peng XH, Chen P, Tang SQ (2012) Synthesis and characterization of a degradable composite agarose/HA hydrogel. *Carbohydr Polym* 88:1445–1452