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pH-sensitive interpenetrating network hydrogels based on chitosan derivatives and alginate for oral drug delivery

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ABSTRACT

Methoxy poly (ethylene glycol) grafted carboxymethyl chitosan (mPEG-g-CMC) and alginate were chosen as the constituents of hydrogel beads for the construction of an interpenetrating polymeric network matrix. A contrast study between the mPEG-g-CMC hydrogel and mPEG physically mixed with CMC hydrogel was carried out. Bovine serum albumin (BSA) as a model for a protein drug was encapsulated in the hydrogel network, and the drug release properties were studied. The hydrogels prepared by these two methods maintained good pH sensitivity; the loading capacity of the mPEG-g-CMC/alginate hydrogel was enhanced in comparison with that of the hydrogel prepared by physically mixing mPEG. The burst release of the protein was slightly decreased at pH 1.2, while the release at pH 7.4 was improved, suggesting that the mPEG-g-CMC/alginate pH-sensitive hydrogel will be promising for site-specific protein drug delivery in the intestine.

1. Introduction

In recent years, some attention has been focused on chitosan and alginate hydrogels as well as their use in implants and controlled-release. Chitosan is a cationic polymer polysaccharide consisting of β-(1-4)-2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose units, which is suitable for oral drug delivery owing to its good physical and chemical properties. These properties include biocompatibility, biodegradability, mucoadhesiveness, permeation enhancing effect, pH sensitiveness and ease of chemical modification as a result of the presence of reactive amine and hydroxyl groups (George & Abraham, 2006). However, the application of chitosan suffers severe limitations because of its poor solubility both in water and organic solvents due to its rigid crystalline structure. To overcome this deficiency, a variety of graft copolymers of chitosan were synthesized and classified as hydrogels (Chen & Wang, 2001; Kennedy & Machnigh, 1995; Yang et al., 2008; Yao et al., 2003), drug delivery systems (Dong & Doo, 1999; Jayakumar, Reis, & Mano, 2006) and surfactants (Ngimhuang, Furukawa, Satoh, Furuike, & Sakairi, 2004). Moreover, some chitosan derivatives have been prepared, among of which carboxymethyl chitosan (CMC) was studied by several authors due to its excellent properties (Chen, Tian, & Du, 2004; Chen, Wu, Mi, & Sung, 2004). It has been reported that CMC having carboxymethyl substituent on some or both the amino and primary hydroxyl sites of the glucosamine units of the chitosan structure is non-toxic, either in vitro or in vivo, in oral or subcutaneous treatments (Kennedy, Costain, McAlister, & Lee, 1996). The carboxylic groups in CMC are expected to decrease the swelling property at relative lower pH value (pH 1.2), whereas increase the swelling property at pH 7.4. Also, some investigations show that the above polymers have the ability to protect protein and peptide drugs from some protease enzymes. However, one shortcoming of CMC is that the ionization degree of carboxylic groups decreases in acidic conditions. A great deal of the hydrogel appears in acidic conditions due to strong hydrogen bonds, which can only be dispersed by strong stirring. The introduction of methoxy poly (ethylene glycol) (mPEG) into chitosan may overcome this disadvantage because mPEG can destroy the strong inter-or intra-molecular hydrogen bonds (Dong, Feng, Qi, Li, & Deng, 2008).

At present, PEGylation of chitosan derivatives is reported by several authors to increase its aqueous solubility. It is well known that PEG as one of the most suitable graft-forming polymers has been employed extensively in pharmaceutical and biomedical fields in the forms of microspheres, nanoparticles or polymers. Its outstanding physicochemical and biological properties are obvious, including solubility both in water and organic solvents, non-toxicity, good biocompatibility and biodegradability and absence

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of antigenicity and immunogenicity. The attachment of PEG to chitosan can increase its solubility and in turn its ability to be processed and applied (Jeong, Kim, Jang, & Nah, 2008). Several publications have reported PEGylation of chitosan with PEG of different molecular weight to improve the chitosan's solubility (Bhattarai, Matsen, & Zhang, 2005; Dal et al., 2000; Prego et al., 2006; Zhang et al., 2007). Dong et al. (2008) prepared the OCMCS-g-mPEGs using aldehyded mPEG and *O*-carboxymethyl chitosan (OCMCS) and indicated that the graft copolymers could be dissolved in water over a wide pH range. Jeong et al. (2010) also studied the doxorubicin hydrochloride (DOX)-incorporated nanoparticles using CMC-PEG by the reaction between the positive amine groups and the carboxymethyl group, suggesting that DOX-incorporated nanoparticles are candidates for antitumor delivery system compared to DOX alone. Moreover, alginate/CMCs-g-AAs (sodium acrylate) hydrogel microspheres coating with the copolymer chitosan grafted PEG (Cs-g-PEG) clearly exhibited their pH responsive nature due to their swelling properties both in the simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). This investigation also indicated that these microspheres had a consistent swelling pattern, high entrapment efficiency and promising sustained release profiles of the model protein drug (El-Sherbiny, 2010). Furthermore, mPEG-g-Cs would also be a promising water soluble wall material or film for the food industry (Peng, Xiong, Li, Chen, & Zhao, 2010).

In this study, methoxy poly (ethylene glycol) (mPEG) grafted CMC (mPEG-g-CMC) was synthesized, characterized and was selected together with alginate to construct the matrix of an interpenetrating network as pH-sensitive hydrogel for oral drug delivery. Alginate, a polyanionic copolymer of mannuronic and guluronic sugar residues is a pH-sensitive polymer which is nontoxic and biodegradable at high pH value when administered orally. Alginate possesses a unique ability to form hydrogels under mild pH and temperature conditions in the presence of divalent cations. Calcium is the most commonly used cation to induce alginate gel formation. The inherent biocompatibility of most alginates makes these materials particularly attractive for biomedical applications including drug delivery, tissue engineering and implantation of protected living cells (Pawar & Edgar, 2012). Several studies have shown that the biological activity of protein drugs can be retained in the calcium-crosslinked alginate encapsulation process (Hari, Chandy, & Sharma, 1996; Murata, Kodama, Isobe, Kofuji, & Kawashima, 2009; Wheatley, Chang, Park, & Langer, 1991). However, the disadvantage of using calcium-crosslinked alginate for protein drug delivery is its macroporous structure and possible rapid dissolution at intestinal pH, which may cause low entrapment efficiency and burst release of core substances (Lin, Liang, Chung, Chen, & Sung, 2005). To overcome this limitation, mPEG-g-CMC was used here to form the interpenetrating polymeric network. Furthermore, bovine serum albumin (BSA) was chosen as a model of protein drug to assess the potential of mPEG-g-CMC co-gelled with calcium alginate (mPEG-g-CMC/alginate) as carriers of a pH controlled release system. For comparison, both mPEG-g-CMC/alginate and CMC/alginate hydrogels with mPEG added by physical mixing method were prepared. The morphology of the beads, the swelling characteristics and the release profiles of the model drug from these carriers in simulated gastric and intestinal medium were investigated.

2. Experimental

2.1. Materials

CMC was acquired from Aoxing Co. Ltd. (Zhejiang, China). mPEG (M_w = 2000 g/mol) were purchased from Fluka (Germany). Sodium alginate of high viscosity (280 cps for a 1% solution at 25 °C) was purchased from Jingyan Co. Ltd. (Qingdao, China). Calcium chloride, phosphate buffered saline (PBS) were purchased from Kewei Co. Ltd. (Tianjin, China). BSA was purchased from Dingguo Biotechnique Co. Ltd. (Beijing, China). All other chemicals and reagents used were of analytical grade.

2.2. Synthesis of mPEG-g-CMC

Firstly, the hydroxyl group of mPEG was modified to the aldehyde activated form (mPEG-CHO) as shown in Scheme 1. 10 g mPEG was dissolved in 30 ml dimethyl sulfoxide (DMSO) and 5 ml acetic anhydride was added to this solution. The mixture was stirred for 24 h under a nitrogen atmosphere. The resulting solution was precipitated by cold diethyl ether and filtered; the white powder thus obtained was dried in vacuum oven for 24 h. The acetalization degree of mPEG-CHO was determined by the hydroxylamine hydrochloride method (Martwiset, Koh, & Chen, 2006). The mPEG-g-CMC copolymer was then synthesized using Schiff's base method (Scheme 1). 5 g CMC was completely dissolved in a 50 ml mixture of distilled water and methanol (4:1, V/V) by stirring and a suitable amount of mPEG-CHO (with a molar ratio of 1:1.1, 1:1.3, 1:2.5, 1:9.5 to the amino groups of CMC) was added and stirred by mechanical stirrer at 100 rpm at room temperature for 24 h. Reduction was carried out by adding potassium borohydride at a molar ratio of 7.5:1 (KBH_4:mPEG-CHO) to the reaction mixture and stirred for 72 h, the solution was concentrated with a rotary evaporator for about 30 min to remove methanol, followed by dialyzed against distilled water for 4 days using dialysis tube (weight cut-off 12–14 kDa). The dialyzed solution was lyophilized to obtain the product. The chemical structure of the product was determined by proton nuclear magnetic resonance (Varian INOVA 500 MHz, USA) using D_2O as the solvent.

The percentage value of the carboxyl and amino content of CMC were determined as 81% and 62%, respectively by the potentiometric determination method (Muzzarelli, Tanfani, Emanuelli, & Mariotti, 1982). The viscosity molecular weight of CMC was between 150 and 170 kDa using Ubbelohde viscometer (Xu, Mao, Liu, Zhu, & Sheng, 2006).

2.3. Determination of degree of substitution (DS) of mPEG-g-CMC

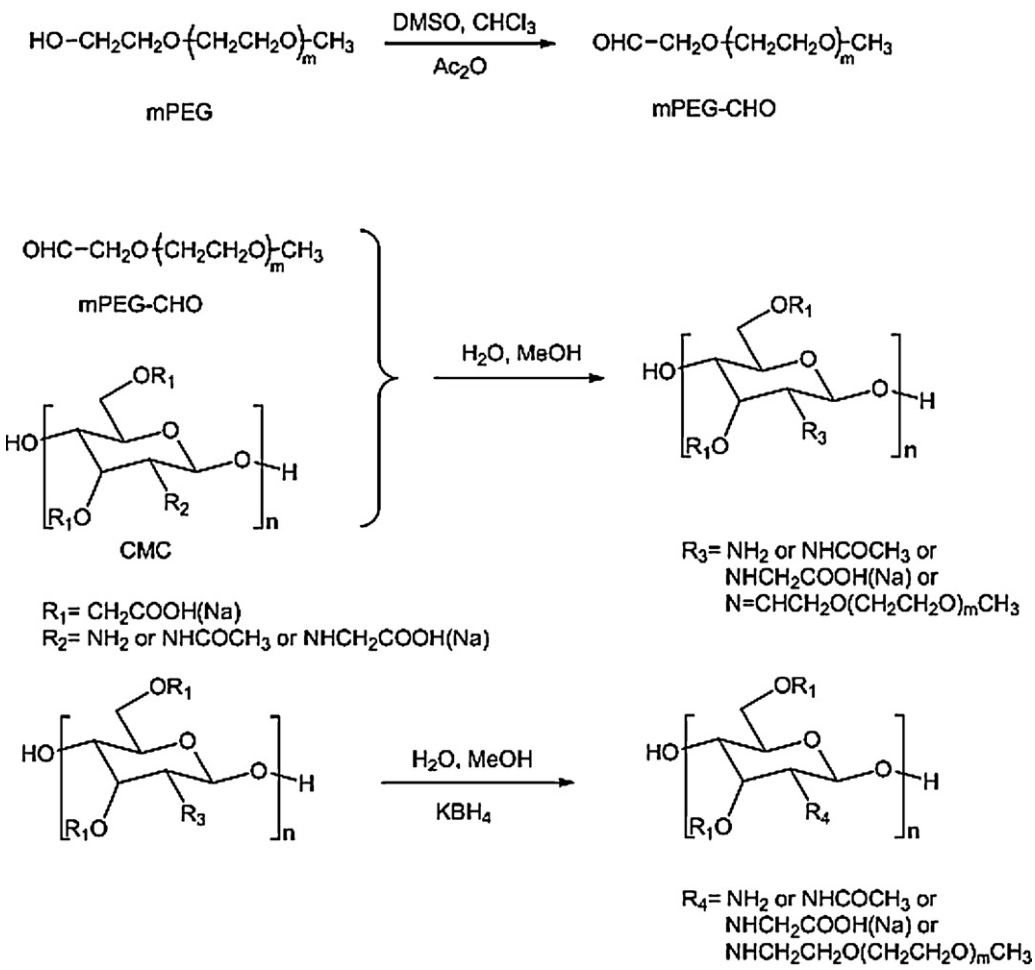
The content of mPEG units in a copolymer was determined by a colorimetric method based on the partitioning of a chromophore present in ammonium ferrothiocyanate reagent from the aqueous to a chloroform phase in the presence of mPEG (Gorochovceva & Makuska, 2004). 3 ml chloroform, 2 ml ammonium ferrothiocyanate reagent and 0.3 ml of a copolymer (0.02 g/10 ml water) solution was mixed vigorously for 30 min. The phases were separated by centrifugal method at 2000 rpm for 2 min. The absorbance of the lower chloroform phase was recorded at 510 nm by UV spectrophotometry method.

The content of mPEG units (mPEG %) in a copolymer and the DS (%) of mPEG in the mPEG-g-CMC were calculated as follows:

$$\text{mPEG\%} = \left(\frac{C \times 2000}{m}\right) \times 100\% \quad (1)$$

$$\text{DS} \quad (\%) = \frac{\text{mPEG\%} \times 252}{(1 - \text{mPEG\%}) \times 2000} \times 100\% \quad (2)$$

where C is the concentration of mPEG (M_w = 2000 g/mol) in the sample solution determined from the standard curve (mol/l); m is the sample weight (g) and 252 is an average molecular weight of a monosaccharide residue of CMC (g/mol).



Scheme 1. Synthesis of the mPEG-g-CMC copolymer.

2.4. Viscosity measurement

Reduced viscosities (η_{sp}/c) of the copolymer solutions (0.02 g/10 ml) with respect to the concentrations of mPEG (C_{PEG}, g/l) in water were measured using Ubbelohde viscometer at 20 °C.

2.5. Preparation of beads

2.5.1. CMC/alginate; mPEG-g-CMC/alginate and mPEG/CMC/alginate hydrogel beads

Blank mPEG-g-CMC/alginate hydrogel beads were prepared by dropping the aqueous mixture of mPEG-g-CMC and alginate into a calcium chloride solution. An aqueous solution of mPEG-g-CMC/alginate with 1:1 (wt%; DS = 18.4 mol%) was dropped into a calcium chloride solution (0.2 M) through a 1 ml pinhead and the blank beads were formed immediately followed by continuously stirred for 30 min. The obtained beads were rinsed in distilled water and subsequently dried in the air for 24 h. The mPEG/CMC/alginate (0.5:0.5:1, wt%) and CMC/alginate (0.5:1, wt%) hydrogel beads were prepared by dropping aqueous mPEG/CMC/alginate solution (or CMC/alginate solution) containing the corresponding concentration of mPEG (M_w = 2000 g/mol) as the mPEG-g-CMC copolymer into the calcium chloride solution in the same method.

2.5.2. Drug loaded beads

BSA with a final concentration of 1% (w/v) was added to the initial aqueous mPEG-g-CMC/alginate solution with continuous stirring. This solution was used for preparation of BSA-loaded beads by the same procedure described in Section 2.5.1.

2.6. Characterization of beads

The swelling behavior represents one of the most significant characteristics that control the drug release rate from hydrogels. The compact degree of the hydrogel beads will have significant effects on the swelling behavior and the drug release properties in aqueous solutions. By means of scanning electron microscopy (SEM), we can analyze the apparent characteristics of the dry beads made by different methods and judge their compact degrees.

2.6.1. Scanning electron microscopy (SEM)

The shape and surface characteristics of freeze-dried beads were determined by SEM. The beads were sputter-coated with Au using a vacuum evaporator and examined using a scanning electron microscope (Nova NanoSEM, FEI, USA).

2.6.2. Swelling studies

The swelling studies of blank CMC/alginate, mPEG-g-CMC/alginate and mPEG/CMC/alginate hydrogel beads were determined by immersing them in a dry state into a conical flask containing 50 ml of release medium that incubated at 37 ± 0.1 °C under constant shaking at 100 rpm. Three release medium were employed to carry out the swelling tests in the solutions of pH 1.2 (0.1 M HCl, simulated gastric fluid, SGF), pH 6.8 (PBS, simulated small intestinal fluid, SIF) and pH 7.4 (PBS, simulated colonic

fluid, SCF). According to the report (Lin et al., 2005), dry beads were firstly swollen in pH 1.2 solution (SGF) for 2 h and then were transferred to pH 6.8 (SIF) and kept for 3 h. Subsequently, they were transferred to the buffer solution at pH 7.4 (SCF) until complete dissolution was obtained. At specific time intervals, samples were removed from the swelling medium and blotted with a piece of paper towel to absorb excess water on surface, and then the beads were weighed.

The swelling ratio (SR) was calculated by the dynamic weight change of the beads with respect to time using the following expression:

$$\mathrm{SR} = \frac{W_s - W_d}{W_d} \quad (3)$$

where W_s is the weight of the beads in the swollen state and W_d is the initial weight of the dry beads. Each experiment was repeated three times.

2.6.3. Drug release studies

The loading capacity (LC) was calculated from the difference between the amount of BSA initially used to prepare the beads and that of the non-associated BSA residues divided by the total mass of beads.

$$\mathrm{LC}\ (\%) = \frac{\text{total amount of BSA} - \text{free BSA in supernatant}}{\text{total weight of beads}} \times 100\% \quad (4)$$

In vitro release studies of the drug loaded beads were also performed in SGF, SIF, and SCF. The prepared BSA loaded beads were placed in conical flask containing 50 ml of the release medium. The samples were incubated at $37 \pm 0.1\ ^\circ$C under constant shaking at 100 rpm. At predetermined time intervals, 5 ml samples were collected from the release medium and were replaced by an equal amount of fresh medium. The concentration of BSA in the solution was assayed by UV spectroscopy (Hitachi UV2450) at 278 ± 2 nm using a standard curve with correlation coefficient $R = 0.999$.

3. Results and discussion

3.1. Characterization of mPEG-g-CMC copolymer

Fig. 1 shows the ^{1}H-NMR spectra of mPEG-g-CMC. The characteristic proton signals of mPEG-g-CMC appeared in the range of 3.4–3.8 ppm and proton assignment of mPEG-g-CMC was as follows: the peak at about 3.22 ppm was the proton signal of methoxyl of mPEG-g-CMC (Yang et al., 2008) and the peak at 2.02 ppm was the proton signal of acetyl group of CMC, which confirmed that mPEG was successfully grafted onto CMC by this method.

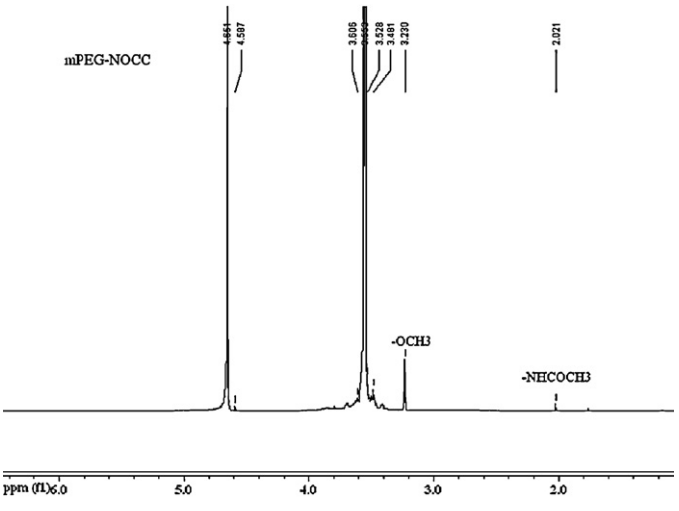


Fig. 1. ^{1}H-NMR spectra of mPEG-g-CMC.

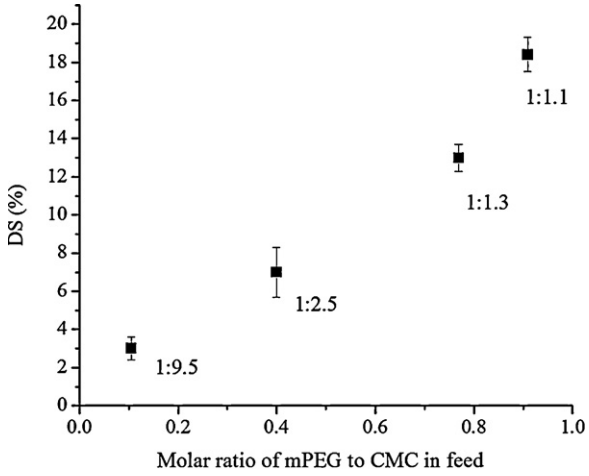


Fig. 2. The degree of substitution (DS) of mPEG-CMC.

3.2. Determination of degree of substitution (DS) of mPEG-g-CMC

The degree of substitution of mPEG moiety can be controlled by the ratio of mPEG to CMC in feed. The results of mPEG subtitution with respect to the molar ratio of mPEG to CMC in feed are shown in Fig. 2.

The molar ratio of mPEG to CMC in feed was 1:9.5, 1:2.5, 1:1.3 and 1:1.1, respectively. As shown in Fig. 2, the DS of mPEG was increased with the decrease of the concentration of CMC in feed. When the ratio came to 1:1.1, the biggest DS of mPEG-g-CMC was 18.4% (mol%). According to the above formulae (1) and (2), with the increase of the mPEG concentration fed into the solutions, the DS of mPEG in mPEG-g-CMC was increased, which was in good agreement with the previous research (Dong et al., 2008). Therefore, in this test, the DS of mPEG-g-CMC was directly depended on the molar ratio of mPEG to CMC in feed. Jeong et al. (2008) reported that PEG grafted chitosan with PEG subtitution of 10%, 15% and 20% were all soluble in various organic solvents. In addition, it was shown that PEG grafted chitosan copolymers with high and moderate amount of PEG chains were soluble in aqueous solutions with wide pH range. Moreover, O-PEGlated chitosan copolymers became water soluble irrespective of pH when DS reached about 15% (Gorochovceva & Makuska, 2004). On basis of these previous studies, mPEG-g-CMC copolymer with DS of 18.4% (mol%) was chosen for the preparation of the pH-sensitive alginate hydrogel.

3.3. Viscosity measurement

Fig. 3 shows the reduced viscosity (η_{sp}/c) of the two sample solutions: the solution of mPEG physically mixed with CMC and the mPEG-g-CMC solution. The reduced viscosities of the both solutions were significantly decreased compared with the viscosity of CMC, which was in good agreement with the reported results (Xu et al., 2006). Compared with the tight hydrogen bonds between the amino and the hydroxyl groups in CMC, the introduction of mPEG weakened the CMC molecular interactions and improved their solubility, which led to the decreased viscosity of mPEG-g-CMC. The grafted mPEG chains separated CMC backbones leading to the disordered characteristic of CMC and therefore decreased the hydrogen bonding. Thus mPEG chain could be thought as a

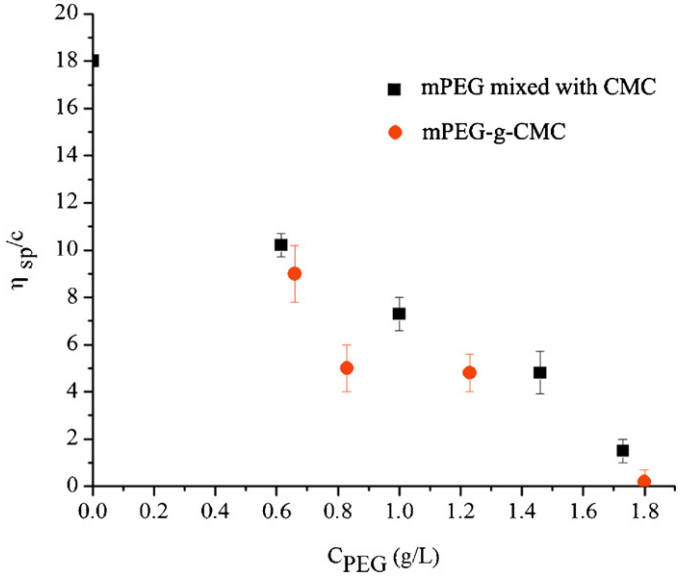


Fig. 3. Reduced viscosity of the copolymer solutions with respect to the concentration of mPEG added by the physical mixing and the chemical grafting methods.

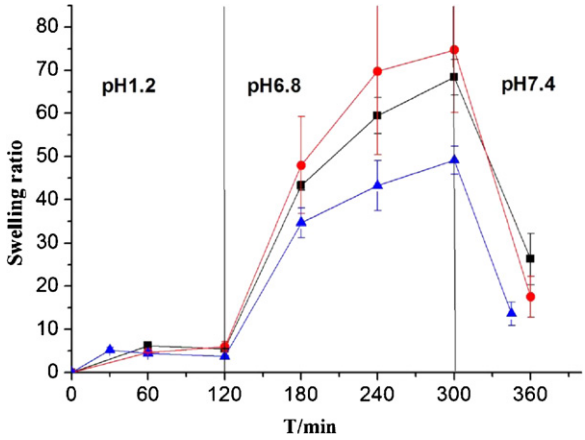


Fig. 5. Influence of mPEG and CMC composition on the swelling ratio of alginate pH-sensitive hydrogel. ( ) CMC:alginate = 0.5:1 (wt%). ( ) mPEG:CMC:alginate = 0.5:0.5:1 (wt%). ( ) mPEG-g-CMC:alginate = 1:1 (wt%); DS = 18.4 mol%.

"molecular windshield wiper" (Gorochovceva & Makuska, 2004). As a result, the lower viscosity of aqueous solution of mPEG-g-CMC copolymer was obtained, which was suitable for making the hydrogel beads and entrapping the protein drugs.

3.4. Morphologies of the hydrogel beads

Fig. 4 shows the surface morphologies of mPEG-g-CMC/alginate, CMC/alginate hydrogel beads with or without mPEG (M_w = 2000 g/mol) addition. The network structure was slightly loosened with the addition of mPEG by the physical mixing method than that without mPEG addition. However, when the mPEG was grafted to CMC, the structure of the mPEG-g-CMC/alginate hydrogel beads became more compact. The reason might be that the physically added mPEG with long soft chains in the hydrogel beads could decrease the cross-link density of the hydrogel network leading to a loose structure of the hydrogel. However, the grafted mPEG chains made the structure of CMC complicated and when the mPEG-g-CMC copolymer was cross-linked by calcium ions with alginate, it enhanced the interaction of hydrogen bonds in the hydrogel network, therefore formed a relatively dense network structure. This structural difference led to the different swellings of the hydrogel beads and the various drug loadings and release behaviors, as described below.

3.5. Swelling characteristics of the hydrogel beads

As shown in Fig. 5, the swelling behaviors of the investigated beads with and without the addition of mPEG by two different methods into the matrix were evaluated. It can be seen from the profiles that the swelling ratios of all the test groups exhibited low values (<5%) in pH 1.2 solution, which could be attributed to the strong hydrogen bond formations between the carboxylic groups and the hydroxyl groups on CMC and alginate leading to the compact structure of the hydrogel beads. The lower swelling ratio of the hydrogel beads in acidic conditions led to a relatively low amount of drug release, which is very important for the protein drugs in case of being destroyed at harsh acidic conditions. After the beads were transferred to the pH 6.8 solution (SIF), the swelling ratios of all the test beads were increased significantly. On the other hand, beads with the addition of mPEG by the physical mixing method exhibited almost the biggest swelling ratio compared to

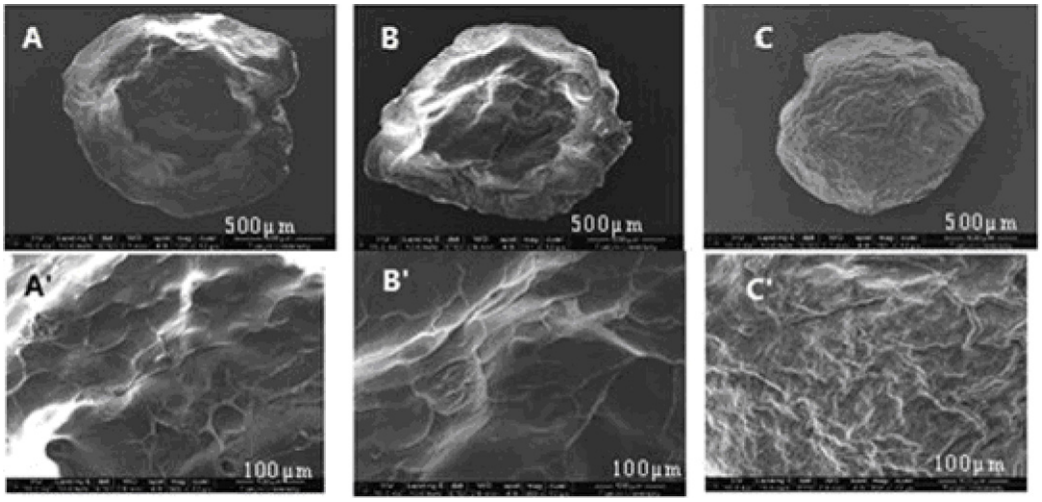


Fig. 4. SEM micrographs of the test beads: (A) CMC/alginate hydrogel beads (0.5:1, wt%); (B) mPEG/CMC/alginate hydrogel beads (0.5:0.5:1, wt%); (C) mPEG-g-CMC/alginate hydrogel beads (1:1, wt%, DS = 18.4 mol%). Original magnifications of A, B and C were 500×, and magnifications of A', B' and C' were 100×.

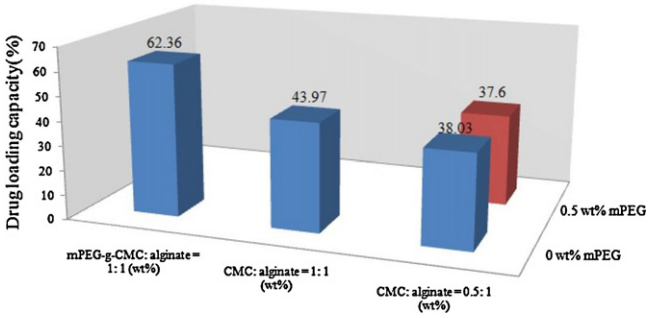


Fig. 6. The loading capacities of hydrogels prepared by different methods.

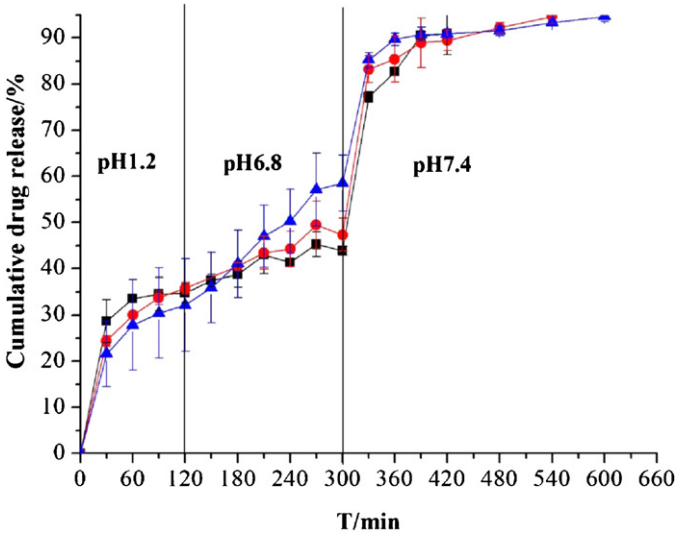


Fig. 7. The release profiles of BSA from pH-sensitive hydrogel prepared by different methods. (■) CMC:alginate = 0.5:1 (wt%). (●) mPEG:CMC:alginate = 0.5:0.5:1 (wt%). (▲) mPEG-g-CMC:alginate = 1:1, (wt%); DS = 18.4 mol%.

those without mPEG and the chemical graft ones. The important increase of swelling ratio at pH 6.8 might be due to the enhanced loose structure of the hydrogel beads with the physical addition of mPEG. When the beads were contacted with water, the physically added mPEG chains were dissolved out, leaving a relative amount of space cavities to hold water. However, the swelling ratio of the mPEG-g-CMC/alginate beads was increased slowly, probably because the chemical grafted mPEG chains, which formed a relatively dense network structure, could not be dissolved out from the copolymer network and the grafted mPEG chains on CMC occupied the network spaces and prevented the hydrogel from absorbing more water. When the hydrogel was subsequently moved to pH 7.4 solution (SCF), the swelling ratio was decreased drastically. In principle, the swelling ratio of the hydrogel beads at pH 7.4 should be enlarged due to the electronic repulsion of the deprotonated carboxylic acid groups on CMC and alginate. However, the gradual degradation of the hydrogel beads after contacted with different environment for about 5 h resulted in the collapse of the hydrogel beads, therefore the swelling ratio was decreased obviously. The collapse of the hydrogel beads at this stage would do contribution to the rapid release of the protein drugs at pH 7.4 environment. The beads with the addition of mPEG by the physical mixing method were collapsed faster than the others at pH 7.4, indicating the rapid diffusion of the mPEG from the network of the hydrogel.

3.6. Drug loaded capacity of the hydrogel beads

As previously reported (Lin et al., 2005), beads prepared with CMC and alginate at weight ratio of 1:1 had better swelling characteristics. With increasing the total concentration of alginate to CMC, the effective crosslinking density of beads increased significantly and a greater amount of drugs were entrapped.

Fig. 6 shows the drug loading capacity of the hydrogel beads prepared by three different methods. As we can see, the loading capacities of BSA which changed from 38.03% to 62.36% were significantly affected by the composition of the hydrogels. For a typical gel composition of CMC:alginate (0.5:1, wt%), the LC was relatively low. However, the protein drug loading was increased to 43.97% by increasing the initial CMC ratio from 0.5 (wt%) to 1 (wt%). On the other hand, the LC was decreased slightly (non significant difference) with the addition of mPEG (0.5, wt%) in the typical gel solution by physical mixing method. As reported previously (Kim & Lee, 1995), intermolecular hydrogen bond could be formed between the electronegative atom of PEG and the amino groups of chitosan during the hydrogel formation. The acid group of BSA and the oxygen atom of the physically added mPEG chain might compete in their interaction with amino groups of CMC, as a result, the possibilities of an interaction between BSA and CMC were reduced. Furthermore, the entanglement of mPEG chains with the CMC molecules hindered the loading of BSA into the hydrogel, leading to a low LC of the protein drugs (43.97%). On the contrary, the LC of the hydrogel beads prepared by mPEG-g-CMC:alginate (1:1, wt%) was significantly improved to 62.36%. The chemical grafted mPEG chains separated the CMC backbones and destroyed the order characteristic of the CMC and drastically decreased the hydrogen bond leading to a low viscosity, which might be responsible for the increase of the LC when the mPEG-g-CMC was used. Vandenberg, Drolet, Scott, and Noue (2001) stated that the highly viscous nature of the gelation medium hinders loading of BSA in the study of chitosan-alginate microspheres. Thus we could draw a conclusion that the mPEG-g-CMC/alginate hydrogel beads possessed higher protein loading capacity than the other preparations.

3.7. Release profiles of BSA from the hydrogel beads

In order to simulate the behavior of the drug release in the gastrointestinal tract, we used the same beads in different solutions, namely the drug loading beads were firstly transferred from the simulated stomach conditions to the small intestine and then to colon conditions by only changing the pH value. This method was also used to investigate the influence of hydrogel swelling properties and degradation behaviors on the drug release using the same beads in different pH solutions.

Fig. 7 shows the BSA release profiles from the test beads prepared by three different methods. It can be seen that all the test beads in three groups showed the burst release of drugs to some extent in the acidic environment in 2 h. The cumulative release amount of BSA from the test beads with addition of mPEG by the physical mixing method and without mPEG addition were 35.8% and 34.7%, respectively. Compared with the other two methods, the drug release from the mPEG-g-CMC was slightly low, about 32% of the encapsulated protein drugs were released in 2 h. The compact structure of mPEG-g-CMC/alginate hydrogel with a high crosslinking density might give the reason for this behavior. After 2 h the beads were subsequently transferred into pH 6.8 solutions (SIF). It can be seen clearly in Fig. 7 that the BSA released from the mPEG-g-CMC/alginate hydrogel was gradually accelerated to about 58% in 5 h, which was higher than that of the test beads with addition of mPEG by physical mixing method (47%) and those without mPEG addition (44%). With the degradation of the hydrogel beads in the final stage, the drug release maintained at approximately

90% in 9 h. The drug release curve showed that a small amount of mPEG addition into the CMC/alginate hydrogel by physical mixing method did not significantly change the properties of the hydrogel and the burst release of drugs at pH 1.2 could not be improved obviously due to the slightly loose network of the hydrogel beads. However, for the mPEG-g-CMC/alginate hydrogel carrier, the burst release of the protein drug was depressed in the harsh acidic environment, whereas a statistical significant release was observed at pH 6.8 and subsequently at pH 7.4 compared to the beads in pH 1.2 solutions. These results suggest that the typical pH sensitive characteristic of the mPEG-g-CMC/alginate hydrogel carrier would be a good candidate for site-specific protein drug delivery in the intestine.

4. Conclusions

In this study, mPEG grafted CMC (mPEG-g-CMC) was synthesized and was chosen together with alginate as the materials to construct the hydrogel matrix of the interpenetrating polymeric network. The hydrogel beads without mPEG addition and those with PEG added by two different methods (physical mixing and chemical grafting) were prepared and investigated. Results showed that the swelling ratio of mPEG-g-CMC/alginate hydrogel beads was decreased in comparison with those without the addition of mPEG and those with mPEG added by physical mixing method, while the drug release behavior was increased. The loading capacity of mPEG-g-CMC/alginate hydrogel was enhanced and the burst release of the protein drug was slightly depressed in the acidic environment in comparison with the one prepared by physical mixing method. The release of the protein drug loaded in mPEG-g-CMC/alginate hydrogel was improved in the alkaline medium. In addition, although a small amount of mPEG addition into the CMC/alginate hydrogel by physical mixing method did not significantly change the properties of the hydrogel, the bioavailability of the mPEG added hydrogel could be improved. These results suggest that the mPEG-g-CMC/alginate as a pH-sensitive hydrogel will be promising for site-specific protein drug delivery in the intestine.

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