Polyelectrolyte Complex Beads Composed of Water-Soluble Chitosan/Alginate: Characterization and Their Protein Release Behavior

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ABSTRACT: Polyelectrolyte complex (PEC) beads were prepared from water-soluble chitosan (WSC) and alginate complex solution with different ratios by dropping method, and all procedures used were performed in aqueous medium at neutral environment. The structure and morphology of the beads were characterized by IR spectroscopy and scanning electron microscopy (SEM). IR spectroscopy confirmed the electrostatic interactions between amino groups of WSC and carboxyl groups of alginate. SEM showed internal section of the PEC bead, which had porous structure compared with compact structure of alginate beads. The swelling behavior, encapsulation efficiency, and release behavior of bovine serum albumin (BSA) from the beads at different pHs were investigated. PEC beads demonstrated different responses to pH from alginate beads. The ratio of WSC to alginate influenced the encapsulation and release of BSA. At pH 1.2, small amount (< 15%) of BSA was released from the PEC beads except AC12. However, at pH 7.4, a large amount (> 80%) of BSA was released from AL in the first 3 h due to the rapid disintegration of the beads, whereas BSA release was retarded from complex beads due to the forming of PEC. The results suggested that the WSC/alginate beads could be a suitable polymeric carrier for site-specific protein drug delivery in the intestine. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 100: 4614–4622, 2006

Key words: drug delivery systems; alginate; PEC; proteins; water-soluble chitosan

INTRODUCTION

Significant advances in biotechnology have resulted in the discovery of a large number of proteins and peptides, which are very effective in disease treatment.[1] However, the intrinsic property of the protein has led to low bioavailability. Recently, natural polysaccharides used as protein carrier have raised tremendous research interests because of their unique advantages, i.e., nontoxic, biocompatible, biodegradable, and rich resources. Also, it is reported that polysaccharide can enhance the absorption of protein drugs.[2]

Chitin and its deacetylated form, chitosan, have received much attention as a material for biomedical and drug delivery applications over the last two decades for their appealing properties.[3,4] Chitosan has good biocompatibility and can be degraded by certain human enzymes.[5] Because of its positive charges, chitosan has the special feature of adhering to mucosal surfaces, which allows paracellular transport across the epithelium.[6] However, chitosan can dissolve in acid, when it was used as an oral drug carrier, the drug releases quickly in the site of stomach. Generally, covalent crosslinking of chitosan was taken to improve its acid stability, but the potential toxicity of free unreacted covalent crosslinkers limited its application. Formation of polyelectrolyte complex (PEC) is an interesting alternative to covalent crosslinking.[7] Polyelectrolyte complexes are formed by mixing solutions of macromolecules with opposite charges. They have been proposed for the design of drug-release systems,[8] wound dressing application,[9] and enzyme and cell support.[10] PEC often exhibits interesting swelling characteristics to the stimulus of pH, ionic strength, and temperature, which are desirable for drug delivery system.

Alginate is an anionic linear polysaccharide with wide application in pharmaceutical industry.[11] It was reported that alginate is nontoxic and biodegradable when given orally.[12] Alginate can form gel with multivalent cations such as calcium ions in aqueous media. The relatively mild gelation process has enabled it an excellent carrier for proteins. Alginate can coacervate with positively charged polyelectrolytes, such as poly-L-lysine, albumin, gelatin, and chitosan. In the alginate–chitosan system, a core-coating model was developed in recent years,[13–15] in which alginate was dropped into chitosan solution. The electrostatic inter-

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action of carboxylic groups of alginate with the amine groups of chitosan results in the membrane formation.[16,17] Although alginate/chitosan microcapsules have been studied a lot, the studies have been limited in a narrow pH region due to the solubility of chitosan.[18]

The purpose of the present study is to explore a novel protein carrier to intestine prepared in neutral environment based on PEC beads. Direct homogenizing of chitosan and alginate solution leads to the formation of unsoluble precipitate. To avoid this, water-soluble chitosan with degree of deacetylation about 50% was prepared. The homogenizing alginate/water-soluble chitosan polyelectrolyte complex was obtained by blending determined volume of the two solutions. The mixture was then dropped into $CaCl_2$ solution to get ionically crosslinked beads. This method provides a mild and favorable neutral incorporation environment for our model protein BSA compared with the weak acid condition used to dissolve chitosan in core-coating model. In the study, PEC beads at various alginate/water-soluble chitosan (WSC) ratio were prepared and characterized by FTIR and SEM. Some aspects of their swelling and release behavior were also discussed.

EXPERIMENTAL

Materials

Sodium alginate was purchased from Shanghai Chemical Reagent Co. (Shanghai, China). The intrinsic viscosity at 25°C was determined to be 550 mL g^{-1}, using a capillary viscosimeter and the M/G ratio was 1.3 measured by ^1H NMR.[19] Chitosan was supplied by Yuhuan Ocean Biochemistry Co., Ltd. (Zhejiang, China). The deacetylation degree of the chitosan was 92%. All other reagents used were of analytical grade. WSC was prepared by acetylating chitosan with acetic anhydride according to literature with some modifications[20]: chitosan (10 g) was dissolved in 250 mL acetic acid solution (2%, v/v) and the solution was mixed with 250 mL methanol. Methanol (50 mL), including 4 mL acetic anhydride was added in drop by drop under stirring to prevent regional gel forming. The stirring was kept for 4 h until a transparent solution was obtained. About 50 mL of alkaline solution(10%, w/v) was poured in to precipitate the water-soluble chitosan. The precipitate was redissolved in water and the pH was adjusted to neutrality by 1*M* HCl. After the solution was dialyzed and concentrated under reduced pressure, it was precipitated by acetone, then dried at 50°C, and kept in desiccators for further use. The deacetylation degree (DD) of WSC was determined to be 52.2% according to titration method,[21] and the average molecular weight (M_w) of WSC measured by gel permeation chromatography (GPC) was found to be 210,000.

Turbidimetric titration and viscosity measurement

The interactions of sodium alginate and WSC were investigated by turbidimetric titration according to the reported method.[22] A solution of 0.1% sodium alginate and 0.2% WSC was prepared at pH 13. Titrant (0.01–0.2*M* HCl) was delivered with a microburette into the solution under gentle stirring, and the pH was monitored by a digital pH meter. Changes in turbidity were monitored at 550 nm with a UV–vis spectrophotometer and the turbidity was estimated by the absorbance.

The viscosity of the polyelectrolete solution with different alginate/WSC weight ratio was measured at 25°C by using ARES-RFS III Rheometer (TA Instruments, USA). Alginate and WSC were dissolved in water to prepare 2% (w/v) solution. Alginate/WSC weight ratio of 2:1, 1:1, and 1:2 were obtained by mixing corresponding volume of the two solutions. These complex solutions did not reveal any sign of phase separation. The samples were degassed and placed for 5 days before measurement. Two parameters, namely, shear stress and shear viscosities, of the aqueous solution of the polymer samples versus shear rate were tested in a steady state.

Preparation of the beads

The alginate/WSC complex solution was prepared by slowly pouring WSC solution (2 wt %) into different volume of alginate solution (2 wt %) with stirring and centrifuged for 20 min under 4000 rpm to remove air bubble. The solution was dropped through a 0.45 mm diameter syringe needle at a drop rate of 1.5 mL/min into 1.0% (w/v) $CaCl_2$ (pH = 6.8) solution with gentle agitation. Spherical beads formed were cured for 4 h at room temperature, then collected by filtration, and washed with water. The beads were dried at room temperature until a constant weight was obtained. Beads containing 1.0% (w/w) BSA were prepared in the same way by dissolving certain amount of BSA in alginate–WSC solution. Three kinds of PEC beads with different alginate/WSC weight ratios of 2:1, 1:1, and 1:2 were prepared and labeled as AC21, AC11, and AC12, respectively. Calcium alginate bead without WSC was labeled as AL.

Characterization

Infrared spectra (IR) of the samples were recorded with a Nicolet-170SX FTIR (USA). The surface morphologies and internal structures of the beads were performed with a Hitachi (Japan) SX-650 scanning electron microscopy (SEM) instrument, dry beads were coated with gold, and subsequently observed and photographed. Gel permeation chromatography (GPC) incorporated a TSP P100 pump (Thermo Sepa-

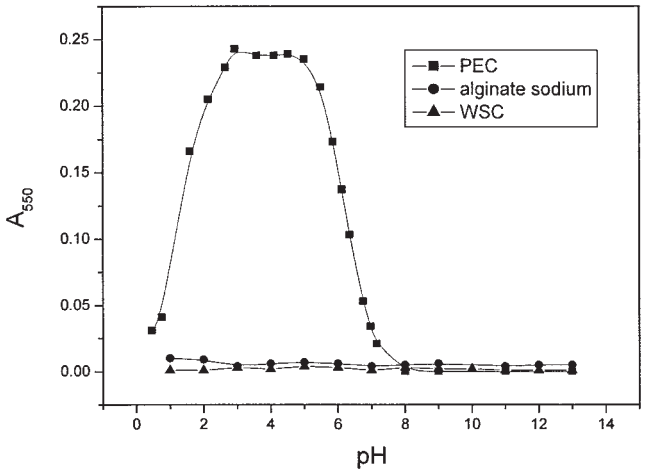


Figure 1 The turbidity titration curves of alginate and WSC and their homogeneous PEC solutions with various weight fractions at 550 nm.

ration products), TSK G5000-PW column, and RI 150 refractive index detector were used for the M_w determination of WSC.

Swelling test

The dry beads (150 mg) containing no BSA were immersed in different pH solutions containing 0.1*M* NaCl for 24 h at room temperature until a swollen equilibrium was reached. The swollen samples were collected by filtration, blotted with filter paper for the removal of the absorbed water on the surface, and then weighed immediately. The degree of swelling (SW) was calculated as follows:

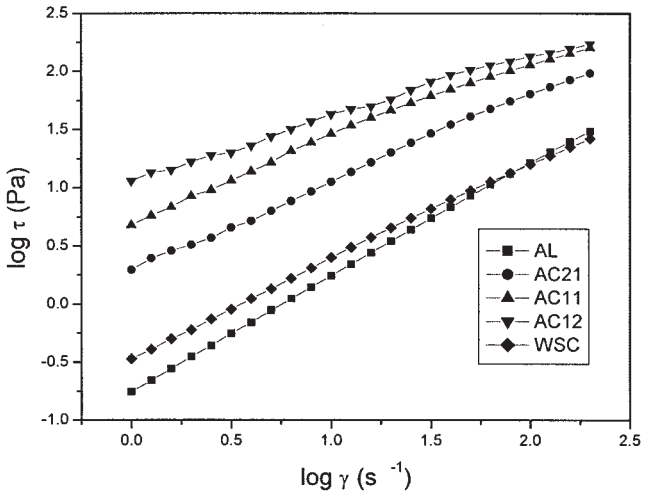


Figure 2 Plots of logτ versus logγ for aqueous solutions of alginate and WSC and their homogeneous PEC solutions at 25°C.

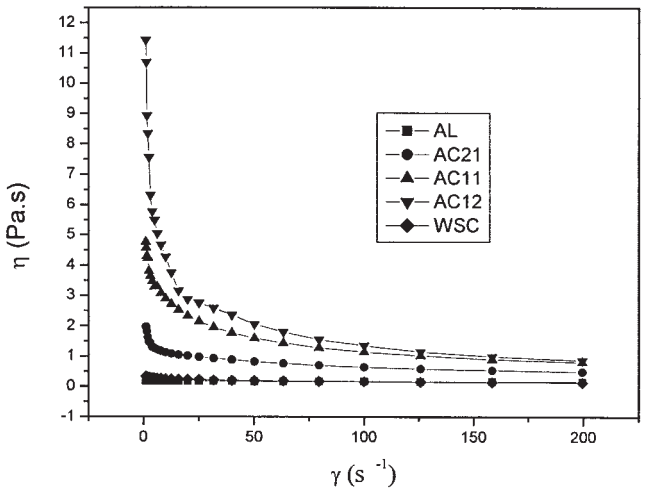


Figure 3 Apparent viscosity versus shear rate for aqueous solutions of two-component polyelectrolytes, alginate and WSC, and their homogeneous complex solutions with various weight fractions.

$$\mathrm{SW} = [(w - w_d)/w_d]$$

where, w and w_d are the weights of the beads in the equilibrium swelling state and in the dry state, respectively.

Determination of calcium content and BSA encapsulation efficiency

The content of calcium in the beads was determined by atomic absorption spectrophotometry (AAS, Hitachi 180–80). PEC beads (50 mg) were suspended in 10 mL concentrated nitric acid and decomposed by heating until 0.5 mL transparent yellow solution was left. The solution was diluted to 250 mL with distilled water and measured by AAS at 422.3 nm. Calcium content in the beads was calculated using calibration curve obtained by using standard $CaCl_2$ aqueous solution.

The drug content in the beads was calculated from the difference between the amount of drug added and the amount of drug in the external aqueous phase, which was determined by Coomassie Brilliant Blue protein assay (Pierce, Inc., New York, NY), using nonloaded beads as basic correction. The BSA encapsulation efficiency (AE) was calculated as follows:

$$\mathrm{AE}(\%) = (A-B)/A\times 100$$

where A was total amount of BSA and B was free amount of BSA.

BSA release from the beads

The in vitro release of BSA from the beads was carried out in 0.1*M* HCl (pH = 1.2) and 0.1*M* phosphate

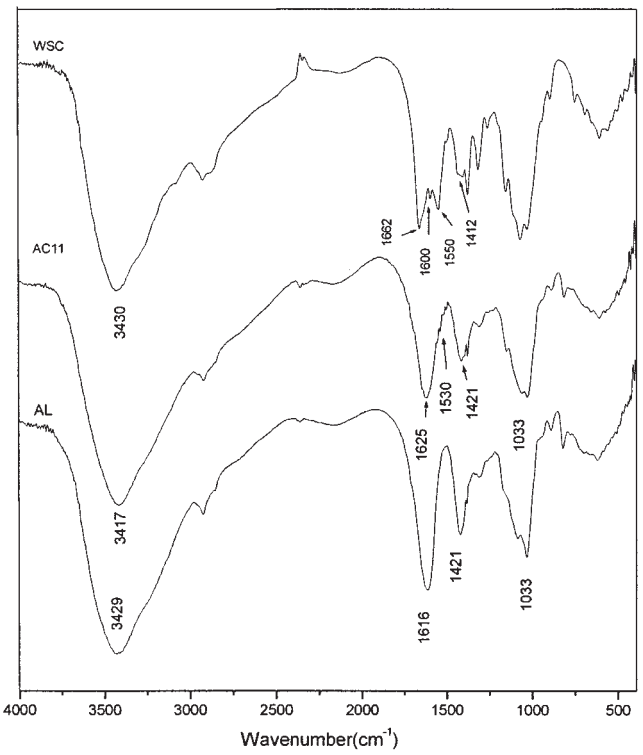


Figure 4 IR spectra of WSC, AC11, and AL beads.

buffered solution (PBS, pH 7.4), respectively. Accurately weighed amounts (100 mg) of the beads were placed in a conical flask containing 100 mL of the buffer and incubated at 37°C at 100 rpm. At selected time intervals, the BSA concentrations in the supernatant were estimated by Coomassie Brilliant Blue protein assay. With each sampling, the same amount of fresh medium was added back to maintain the original volume.

RESULTS AND DISCUSSION

The interaction between WSC and sodium alginate

Chitosan is a polybase with a pK_a of 6.3. The ionization of amine groups increased as the pH decreased. Acetylation of chitosan reduced the number of amino groups on the chain and the positive charge density as well. Chitosan with the degree of deacetylation about 50% can dissolve in water, even in weak basic solution. The electrostatic interactions in the PEC formed by WSC and alginate were depressed because of less amino groups. In addition, this interaction was also controlled by pH. When the pH of the complex solution was above 8.0, the viscosity raised by electrostatic interactions of the complex solution was found to decrease significantly. The turbidimetric titration curves of alginate and WSC and their homogeneous PEC solutions are shown in Figure 1. At high pH (9.0–12.0), the solution was optically clear because of the low charge density of alginate and WSC. However, the turbidity increased greatly when pH was under 7.0, and the highest value of turbidity was in the pH range 3.0–5.0. This could be attributed to the significant charge densities of electrostatic attractions between alginate and WSC in this pH region. Further decrease of solution pH below 3.0 led to the partial disintegration of the PEC, and hence decreased the turbidity. As a contrast, alginate and WSC both showed low turbidity in the tested pH range.

The flow behavior (Fig. 2) and the viscosity (Fig. 3) of the PEC solutions were also studied. From Figure 2, it is observed that all the PEC solutions show non-Newtonian behavior. With the increase of shear rate, the shear stress increases. Figure 3 shows the viscosity curves of the PEC solutions. The viscosity properties of the complex solutions were enhanced when compared with the component alginate and WSC solutions. With increases in shear rate, the viscosity of PEC solutions decreases, showing a shear-thinning behavior. The viscosity of the PEC solution is found to be increased with increased proportion of WSC in the complex. When the mole ratio of alginate and WSC was about 1:1, the sample demonstrated the highest viscosity. The homogeneous interpolyelectrolyte complexation of these two oppositely charged polymers in aqueous solutions through electrostatic interactions may lead to a more expansive conformation of the polymer chains in solution, and enlarge the hydrodynamic volume of the polymer chains.[23] Moreover, the oppositely charged functional groups are expected to

TABLE I
Particle Size, Calcium Content and BSA Encapsulation Rate of the PEC Beads

Sample	Diameter[a] (mm)		Calcium content[b] (%)	BSA encapsulation rate[b] (%)
	Wet	Dry		
AL	2.35 ± 0.09	1.03 ± 0.18	0.266 ± 0.02	37.65 ± 4.1
AC21	2.11 ± 0.05	1.01 ± 0.21	0.245 ± 0.01	45.93 ± 3.0
AC11	3.90 ± 0.14	1.62 ± 0.32	0.234 ± 0.002	37.55 ± 4.6
AC12	4.11 ± 0.11	2.20 ± 0.40	0.223 ± 0.003	25.55 ± 0.13

[a] Data shown are the mean ± standard deviation (n = 10).
[b] Data shown are the mean ± standard deviation (n = 3).

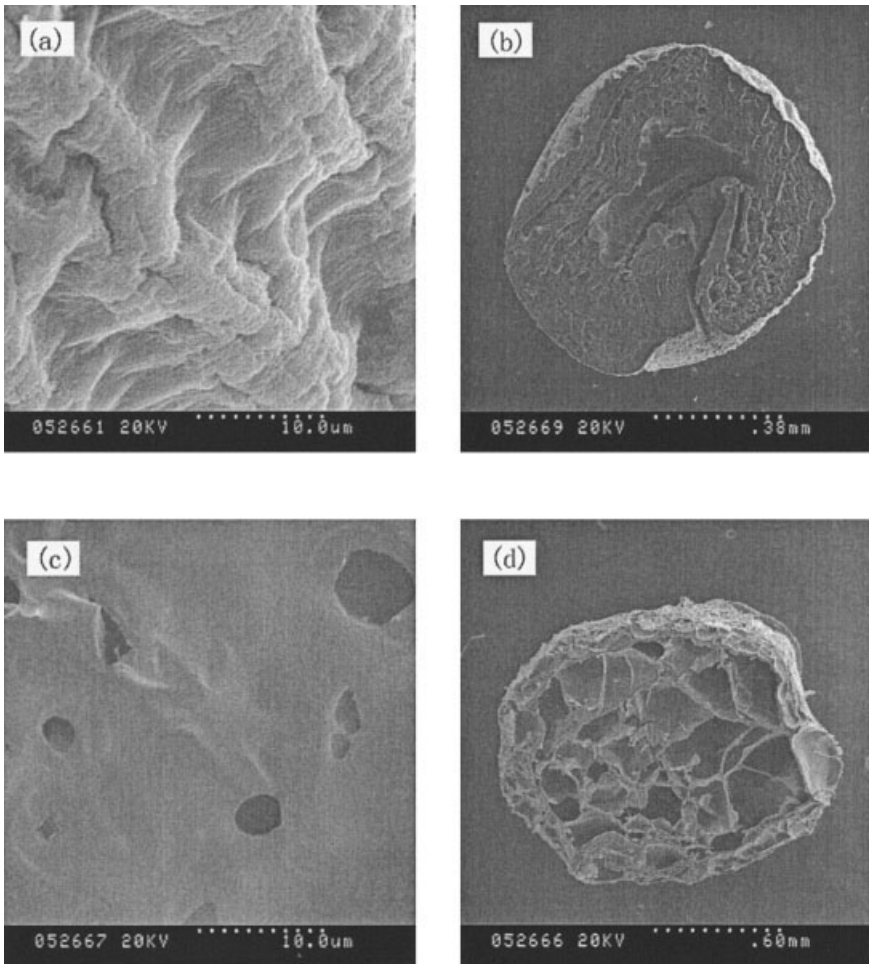


Figure 5 Surface and internal SEM photographs of the beads: (a) surface morphology of AL beads, (b) internal section of AL beads, (c) surface morphology of AC21 beads, (d) internal section of AC21 beads, (e) surface morphology of AC11 beads, (f) internal section of AC11 beads, (g) surface morphology of AC12 beads, and (h) internal section of AC12 beads.

restrict the mobility of the polymer chains by electrostatic interaction, thus improving the stability of chitosan in gastric cavity and alginate in intestine fluid as well.

Structure and morphology of the beads

The IR spectras of the WSC, AL, and AC11 beads are shown in Figure 4. WSC exhibited characteristic absorbing bands of —$CONH_2$ at 1662, 1550, and 1315 cm^{-1}, corresponding to amide I, II, and III, respectively,[24] and the absorption peak of free amino group appeared at 1600 cm^{-1}. These demonstrated that chitosan was acetylated by reacting with acetic anhydride. In IR spectra of AL, characteristic peaks of alginate were shown at 1616 and 1421 cm^{-1}, which can be attributed to the asymmetrical and symmetrical stretching vibration of —COO^-. The spectra of AC11 showed decreased intensity and displacement of the —COO^- group absorption band from 1616 to 1625 cm^{-1}, and a small absorption peak at around 1530 cm^{-1}, which did not appear in either the spectra of alginate or the WSC. This peak was due to a —NH_3^+ group of WSC after the formation of the polyelectrolyte complexes. These evidenced the electrostatic interaction between amino group of WSC and carboxyl group of alginate. Additionally, in comparison with WSC (at 3430 cm^{-1}) and alginate (at 3429 cm^{-1}), the —OH stretching vibration band of the PEC beads was slightly broader and shifted to a lower wave number (at 3417 cm^{-1}), suggesting that intermolecular hydrogen bonds also existed in the beads.

Particle size of the beads in wet and dry state was measured by using a micrometer caliper. The results are listed in Table I, the dry beads were about 1–2 mm. The diameter of the PEC beads was almost doubled when the WSC fraction increased from 33.3% (w/w) to 66.6% (w/w). This change was probably related to the enhanced viscosity of mixture and lower crosslink intensity with calcium ions. The viscosity increased as the content of WSC raised, and higher viscosity caused by stronger electrostatic function can lead to larger

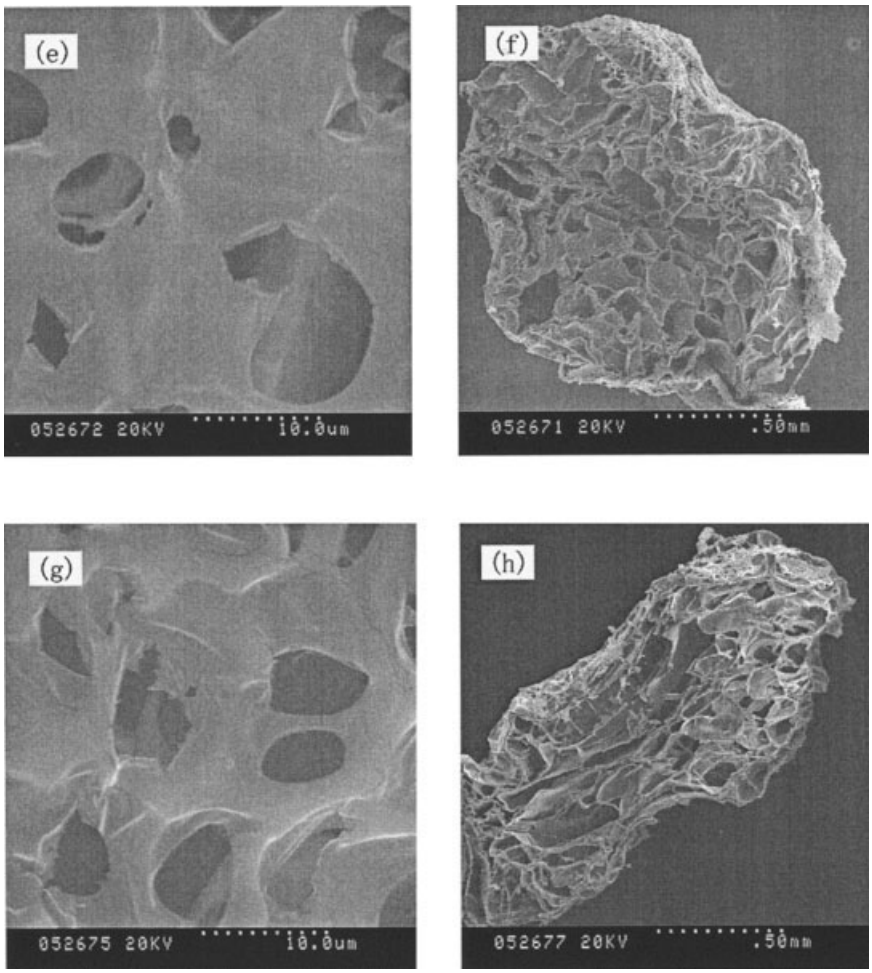


Figure 5 (*Continued*)

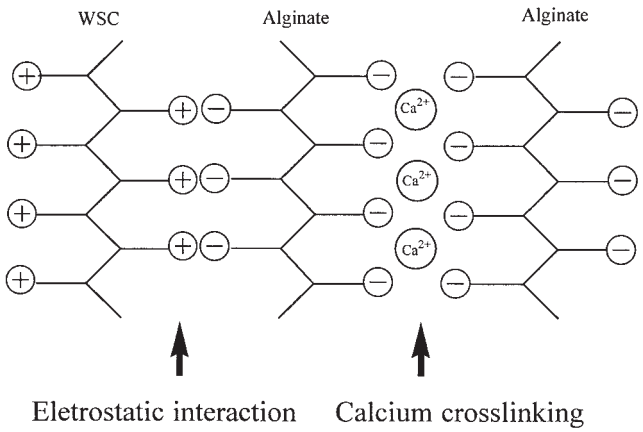
beads.[25] Moreover, the increased proportion of WSC in the PEC decreased the crosslink density with Ca^{2+} (Table I), which should also result in larger bead diameter.

The surface and internal morphologies of AL and PEC beads are shown in Figure 5. The surface of AL exhibited a wrinkle-like appearance and no pores were found [Fig. 5(a)]. The internal morphology of AL displayed a heterogeneous structure and the out-layer is relatively dense [Fig. 5(b)]. The surface and internal morphologies changed significantly because of the incorporation of WSC into alginate. Macropores were observed on both the surface and internal section of the PEC beads [Fig. 5(c)]. AC21 had a porous core, which was surrounded by a thin layer [Fig. 5(d)]. With increased fraction of WSC in PEC beads, the out-layer was gradually disappeared and the beads became more homogenous. Also, more pores were found on PEC beads with less alginate/WSC ratio [Fig. 5(e)–(g)]. The porous structure decreased the mechanical propertied of the PEC beads and led to poor shape of the dry beads [Fig. 5(f)]. AC12 almost lost its shape upon drying [Fig. 5(h)]. The porous structure found in our PEC beads was also found in other polysaccharide-based polyion complexes.[26]

Earlier results indicated that the PEC beads had different structure and morphology from AL beads. The proposed binding mechanism of the PEC beads is schematically showed in Scheme 1.

Effect of pH on the swelling ratio

To characterize the response of PEC beads to the external pH conditions, PEC beads were allowed to swell to equilibrium in 0.1*M* NaCl at various pHs. In Figure 6, the swelling ratio of various samples is plotted as a function of the pH. It is clear that the PEC beads showed different swelling behavior from AL. The swelling degree of AL bead had an increased trend when the pH changed from acidity to neutrality because of the replacement of Ca^{2+} by Na^{+}. Further increase of pH decreased its swelling on the contrary. All the PEC beads exhibited smaller swelling degree than that of AL. In low pH range, the minimum swelling appeared at pH 3. It is known that chitosan has a pK_a value of 6.3, whereas alginate has pK_a values of



Scheme 1. The proposed binding mechanism in the WSC/alginate beads.

3.4–4.4. At pH near 3.0, the amino groups in chitosan are protonated and the carboxyl groups in alginate are ionized; thus, the strong electrostatic attractions between chitosan and alginate restrain the swelling of the PEC beads. At pH 1.0, the increased swelling may be caused by the dissociation of ionic bonds between WSC and alginate as most of the carboxyl groups are charged, and change into their protonated form. On the other hand, at high pHs, most of the amine groups of the chitosan are in the $—NH_2$ form and most of the carboxyl groups of the alginate are in the $—COO^-$ form, and so the electrostatic attractions were weakened in PEC beads, thus increasing the beads swelling. The decrease of swelling at pH 13 was probably caused by the formation of new crosslinks by hydrogen bondings and hydrophobic interactions in the beads.[27] The swelling of the PEC beads was also related to the blend ratios of alginate and WSC. In neutral and basic environment, the swelling ratio of AC21 was higher than that of AC11, and AC12 had the lowest swelling value. This can be attributed to the enhanced electrostatic interaction with the increased fraction of WSC in the beads. The mole ratio of alginate to WSC was about 1.0 in AC12, and the attractions between amino and carboxyl groups were strengthened. However, an opposite trend of swelling was observed at pH 1.0. In this case, the interaction between WSC and alginate was reduced significantly. Swelling was mainly caused by the repulsion of $—NH_3^+$ groups, and so increased fraction of WSC in PEC beads led to high degree of swelling.

BSA encapsulation and its in vitro release

The influence of WSC on BSA loading efficiency is listed in Table I. It can be seen that the BSA loading was improved by incorporating appropriate amount of WSC. The network formed by alginate and WSC could entrap BSA through intermolecular interactions. Further crosslinking with Ca^{2+} resulted in a more tight and ordered network, which was similar to the formation of semi-interpenetrating network (*S*-IPN). However, when WSC ratio was increased to more than 66%, the BSA loading efficiency of AC12 decreased instead. This may be conducted by lower crosslinking degree between alginate and Ca^{2+}, as shown in Table I, leading to the leakage of BSA from the beads. Another reason may lie in different structure of AC12 from AC21. From the study of SEM, it was found that AC12 is more homogenous and porous than AC21.

The release of BSA from different alginate/WSC composition beads were performed in pH 1.2 and 7.4 buffer solution, respectively, which simulated pH conditions in the GI tract. Figure 7 depicts the release profiles of BSA in pH 1.2 HCl. It is obvious that the release rate closely related to alginate/WSC ratio. No burst release was observed except AC12. BSA release in AL beads was lower than 10% after 10 h. AC21 and AC11 have slight higher release rate, but both under 15%. AC12 showed faster release behavior, in the first 2 h, more than 50% BSA was released. As shown in our swelling study, AL has the minimum swelling degree, whereas AC12 has the largest swelling at pH 1.0, which suggested that the release of BSA from the beads in acid condition was related to their swelling behavior. Large swelling can provide enough pore sizes, which accelerate the diffusion of BSA bounded in the beads. The release behavior of BSA in phosphate buffer is shown in Figure 8; quick release of BSA in AL can be seen due to its disintegration in PBS. The formation of PEC between alginate and WSC improved the stability of the bead in PBS because of the electrostatic interaction and intermolecular hydrogen bonds, thus retarding the BSA release. Only 50% BSA was

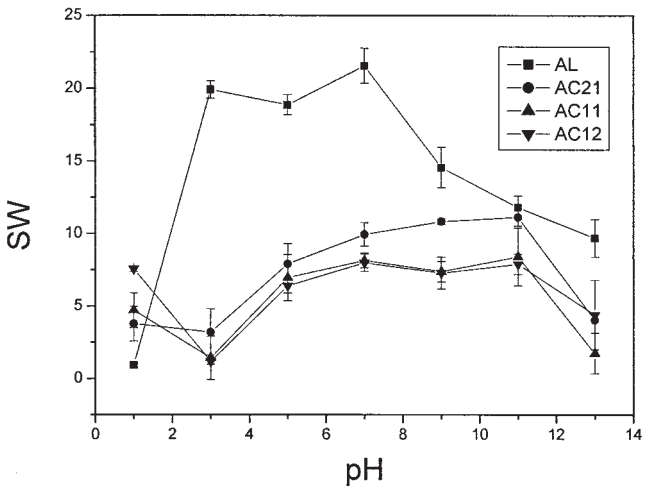


Figure 6 Swelling degree of AL and AC11 beads at various pHs containing 0.1*M* NaCl. Data shown are the mean ± standard deviations ($n = 3$).

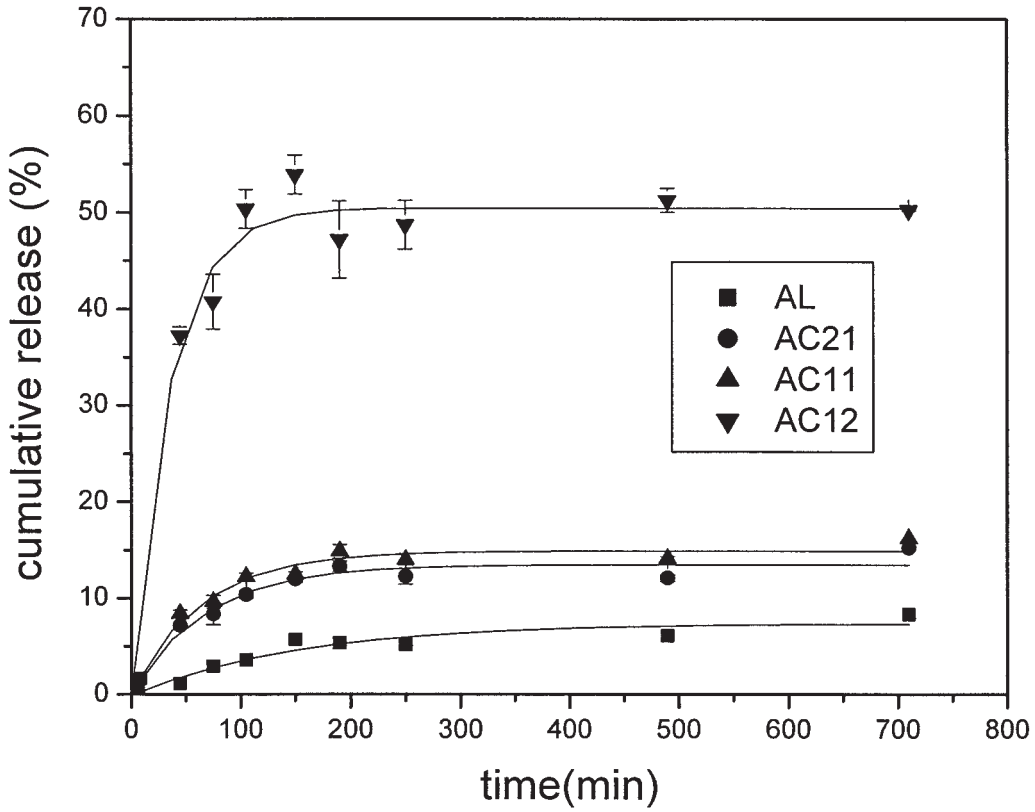


Figure 7 Release profiles of BSA from the beads in pH 1.2 HCl. Data shown are the mean ± standard deviations (n = 3).

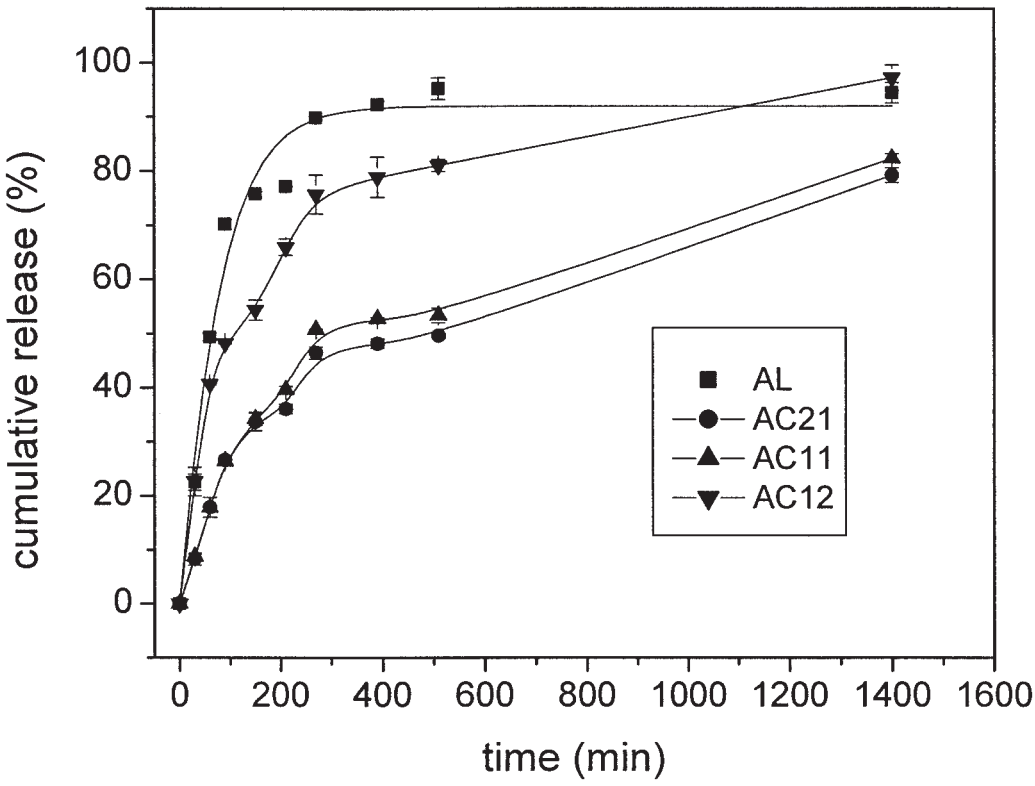


Figure 8 Release profiles of BSA from the beads in phosphate buffer (pH 7.4). Data shown are the mean ± standard deviations (n = 3).

released after 10 h's of cultivation in AC21 whereas 90% BSA was released from AL in the same period. Although AC21 has greater loading efficiency than AC11 and AC12, it is showing smaller release. This can be explained by different structure of the PEC beads. As shown in SEM study, AC21 has a core-shell structure, whereas AC12 showed homogeneous sponge-like morphologies. BSA can easily escape from AC12 through pore channels on the surface. These results suggested that compared with either polymer alone, WSC/alginate PEC beads with certain blend ratio could be a suitable polymeric carrier for protein drug delivery in the intestine, together with the prevention of the release at the gastric level.

CONCLUSIONS

From this study, we demonstrate that the swelling and release behavior of AL bead can be modified through blending with WSC. The WSC/alginate ratio plays a key role in release experiment. When the ratio is below 1, the PEC beads provide desired protective effect on protein at low pH and prolonged release at high pH environment. These results enable WSC/alginate PEC beads to be used as oral sustain delivery system for protein drugs.

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