pH-Sensitive Alginate/Soy Protein Microspheres as Drug Transporter

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ABSTRACT: The complex microspheres based on alginate (AL) and soy protein isolate (SPI) were prepared by solution blending and then Ca^{2+} crosslinking, and their function as drug carrier was explored as well. The effects of composition on the structures of microspheres were studied, and the XRD results proved the miscibility between components. Meanwhile, FTIR results suggested that such miscibility was driven by strong hydrogen bonding. Especially, the complex microsphere with equal content of AL and SPI had the best miscibility by morphological analysis, shown as a smooth and uniform surface of SEM images. The controlled release function of the complex microspheres was verified using theophylline as a drug model, that is, the swelling and drug release were affected by pH conditions and showed obvious differences under given pH of stomach, intestine, and colon. Moreover, the intestine and colon may be optimal site for prompt release of drugs. Except for the attribution of AL component to pH sensitivity, the complex microspheres also inherited the bioactivity of SPI component, which may lower irritants of drug to the tissues in body. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 106: 1034–1041, 2007

Key words: soy protein; alginate; microsphere; blend; drug-delivery system

INTRODUCTION

A wide range of biocompatible natural polymers such as polysaccharides and proteins, such as chitosan,[1] alginate (AL),[2] cellulose,[3] soy protein,[4] zein,[5] casein,[6] and so on, are potentially available as the biomedical materials,[7] and especially has been developed as the carrier of oral drugs. Microspheres are currently a typical form of drug carrier.[8] The drugs are encapsulated and taken by microspheres to mask taste and odor, to enhance stability, to improve gastrointestinal tolerance, and to provide sustained release after oral administration.[9] Depending on the nature of the components, the microspheres may be sensitive to pH variance of tissues in the human body.[10] Furthermore, the loaded drugs can be controlled-released as the microsphere swells under given pH, and settles at the target sites of effective absorption, and prolongs duration of activity by selectively adhering of microsphere.[5] In addition, the bioactivity of other components in microspheres may facilitate the curative effect or reduce the side effect of drugs.[4] Especially, the miscible blends can be used to adjust the behavior of drug release depending on the properties of components as well as the compositions in whole blend and the interaction between components, and especially produced the function of the pulsatile chronotherapeutics and the controlled profiles of drug release.[11]

Biocompatible ALs can be ionically crosslinked in the presence of multivalent cations[12] and induced the gelation, which was already used to produce many kinds of biomedical materials, such as hydrogel,[13] microcapsule,[14] microsphere,[5,8–10] membrane,[15] and fiber.[16] The AL-based microspheres crosslinked with Ca^{2+} exhibited controlled-release function due to the sensitivity of Ca^{2+}/COO^{-} linkage to pH and other ion.[17,18] It is well known that the linkage between Ca^{2+} and COO^{-} can be only stable in acidic condition. Thus, the AL-based microspheres can swell and even collapse with increasing pH. Such pH sensitivity is expected to control the drug release at alvine and colonic sites.[17] To enhance the pH sen-

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sitivity and functions of AL-based microspheres, other biocompatible polymers are introduced by a simple and effective method of blending. For example, the carboxymethylated chitosan was mixed with sodium AL, producing a complex microsphere by Ca^{2+} crosslinking, by which the release ratio of bovine serum albumin (BSA) encapsulated was under the control of pH.[18] On the basis of the polyelectrolyte complexation between positive and negative ions, the chitosan and poly(lysine) were blended respectively with AL, forming the drug carriers of microsphere.[19]

Soy protein is a plant protein to favor the health of human being, and can be thermoplastic-processed and solvent-cast as biomedical materials. The possible benefits of soy protein include lowering cholesterol, anticarcinogenic effects of Bowman-Birk (BBI), and protective effects against obesity, diabetes, irritants of the digestive tract, bone, and kidney diseases.[20] On the basis of the bioactivity of soy protein, the soy protein plastics prepared by melt-processing[21] and further modification of chemical crosslinking[22] showed a great potential applications as tissue engineering scaffold[23] and drug-delivery system.[22] Additionally, the soy protein-based membrane was also used as drug carrier and wound dressing material.[24] To further improve the biofunction and properties, the blending with other biocompatible polymers, such as chitin[25] and chitosan,[26] cellulose,[27] and poly(ethylene glycol),[28] was attempted, and especially produced a new form material of hydrogel for drug delivery.[28] The blend materials had enhancing mechanical properties[25] and inherited the bioactivities of added components,[26] which benefited the application of tissue engineering.[26] However, as we know, the microsphere of drug carrier containing soy protein has not been developed so far.

The objective of this work is to offer a complex microsphere based on AL and SPI as a drug carrier, which could combine the pH sensitivity of AL with the bioactivity of SPI, by solution blending, and then Ca^{2+} crosslinking. Subsequently, the effects of compositions on microsphere structure and the interaction between components were investigated. As a potential drug carrier, the swelling test of complex microspheres under given pH conditions was carried out. Furthermore, the theophylline as a drug model was encapsulated by microspheres to study the release behaviors in simulated pH conditions of stomach, intestine, and colon.

EXPERIMENTAL

Materials

Commercial soy protein isolate (SPI) was purchased from DuPont-Yunmeng Protein Technology (Yunmeng, China). The weight–average molecular weight (M_w) of SPI was determined by multi-angle laser light scattering instrument (MALLS, DAWN® DSP, Wyatt Technology, USA) equipped with a He–Ne laser (λ = 632.8 nm) to be 2.05×10^5.[29] The original moisture content, protein content, and amino acid compositions of SPI have been investigated and detailed in our previous work, and the protein content of more than 90 wt % and 18 diverse amino acids were determined.[29] Sodium AL was purchased from Guoyao Chemical Regents (Shanghai, China). Theophylline (1,3-dimethylxanthine) was supplied by Xingrui Biological Regents (Wuhan, China). The other reagents of analytical grade were obtained from Shanghai Chemical (Shanghai, China). All the reagents were used as received.

Preparation of complex microspheres

SPI was dispersed in distilled water at room temperature, and then basified with 10 wt % NaOH aqueous solution to produce a viscous liquid of pH 9–10 containing 3 wt % SPI. Meanwhile, AL was also dissolved in distilled water to obtain a solution containing 3 wt % AL. Subsequently, the resultant SPI and AL solutions were mixed by the weight ratio of 1 : 3, 1 : 1, and 3 : 1, respectively. In the mixing solution, the weight ratios of SPI vs. AL were consistent with the weight ratios of SPI and AL solution as mixing, namely the SPI content in the total weight of SPI and AL were 25, 50, and 75 wt %, respectively. The mixing solutions were mechanically stirred at room temperature for 30 min to result in a homogeneous dispersion of SPI and AL components. At last, the complex microspheres formed by injecting the mixing solutions into 10 wt % $CaCl_2$ aqueous solutions using the syringe equipped with 9# nozzle in a speed of 10 mL min^{-1} as well as crosslinking —COOH groups in AL and SPI molecules in the aqueous solution containing Ca^{2+} for 30 min. The obtained microspheres were washed with distilled water to remove the free ion attached onto the surface and existed into internal holes. According to the SPI content in the solid, the microspheres were coded as SA-1 (25 wt % SPI), SA-2 (50 wt % SPI), and SA-3 (75 wt % SPI). The typical panorama of microspheres was shown as an example of SA-3 in Figure 1. At the same time, the pure AL microsphere was also prepared only from the AL solution according to the process mentioned earlier. The diameters range of the microspheres in dry state was determined as 1000 ± 100 μm after the evaporating of water.

The drug-loaded microspheres were prepared as follows, and the theophylline as model drug were used. The theophylline was mixed with the SPI/AL mixing solution with weight ratio of 1 : 1, in which

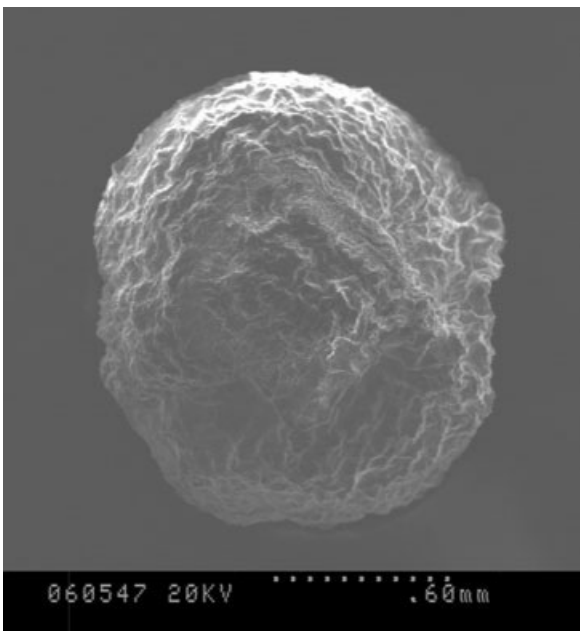

Figure 1 The panorama of SA-3 microsphere as an example of all the blend microspheres and pure AL microsphere.

the SA-2 microsphere with best miscibility was used while the content of theophylline is 20 parts with regards to the 100 parts of the whole solid of AL and SPI. Subsequently, the mixing solution containing theophylline was crosslinked by Ca^{2+} and then produced the drug-loaded microspheres. All the drug-loaded microspheres were washed by distilled water to remove the drug attached onto the surface and then dried before the study of drug release.

Characterization

Fourier transform infrared (FTIR) spectra were recoded on a FTIR 5700 spectrometer (Nicolet, USA). The AL and SPI powders were taken with the method of KBr platelet and scanned in the range of 4000–400 cm^{-1}. At the same time, the freeze-dried microspheres were measured by using Smart OMNT reflect accessories in the range of 4000–700 cm^{-1}.

X-ray diffraction (XRD) patterns were recorded on D/max IIIA X-ray spectrometer (Rigaku Denki, Japan) using Cu Kα (1.54056 Å) radiation (35 kV, 30 mA). All the powder samples were mounted on a sample holder and scanned from 2° to 60° in 2θ at a speed of 10° min^{-1}.

The surface and cross-section of microspheres were photographed using an S-570 scanning electron microscope (Hitachi, Japan) with 20 kV as the accelerating voltage. All the microspheres were frozen in liquid nitrogen while some were fractured immediately, and then they were freeze-dried followed by coating with gold for observation.

Swelling tests of complex microsphere

The swelling ratio of microspheres at room temperature was determined by swelling the dried microspheres in HCl solution of pH 1.0 and phosphate buffer solutions of pH 6.8 and 7.4, which respectively corresponded to the pH environments of stomach, intestine, and colon in body. The dried SA-2 microspheres with the best miscibility (~ 1.0 g) were placed in the solutions with different pH for a required period of time. Subsequently, the swollen complex microspheres were taken out and its wet weight were immediately determined after removing the adsorbed water on the surface with a filter paper. The percentage swelling of complex microspheres in the medium was calculated as follows:

$$S = \frac{W_t - W_0}{W_0} \times 100\%$$

where S is the percentage swelling of the complex microspheres at a predetermined time; W_t and W_0 are the weight of swollen complex microspheres at a predetermined time and their initial weight, respectively. An average value of five replicates for each sample was taken.

Drug-release studies

The theophylline was selected for the experiments of drug release because its UV absorption cannot be overlapped with the components of complex microspheres. Similar to the swelling tests again, three pH values of 1.0, 6.8, and 7.4 were adjusted. The drug-loaded SA-2 microspheres were used to study the drug release *in vitro* as follows. The drug-loaded microspheres (~ 0.5 g) were incubated into 45-mL solution with various pH at 20°C. After the given intervals, the 5-mL solution was removed for determining the release content of drug, which was obtained from the absorbance at 286 nm measured on a UV-160A spectroscope (Shimadzu, Japan). Subsequently, the 5-mL fresh buffer solution was supplied to keep the total volume of 45 mL of solutions.

RESULTS AND DISCUSSION

Miscibility between SPI and AL components

Figure 2 shows the XRD patterns of complex microspheres as well as pure SPI powder and AL microsphere. Obviously, both the SPI powder and AL microsphere showed the semicrystalline character with two diffuse peaks. The peaks of the former located at 9.8° and 19.9° of 2θ while those of the latter located at 12.9° and 22.0° of 2θ. However, when two components were blended as complex microspheres, there was no peak in the XRD patterns,

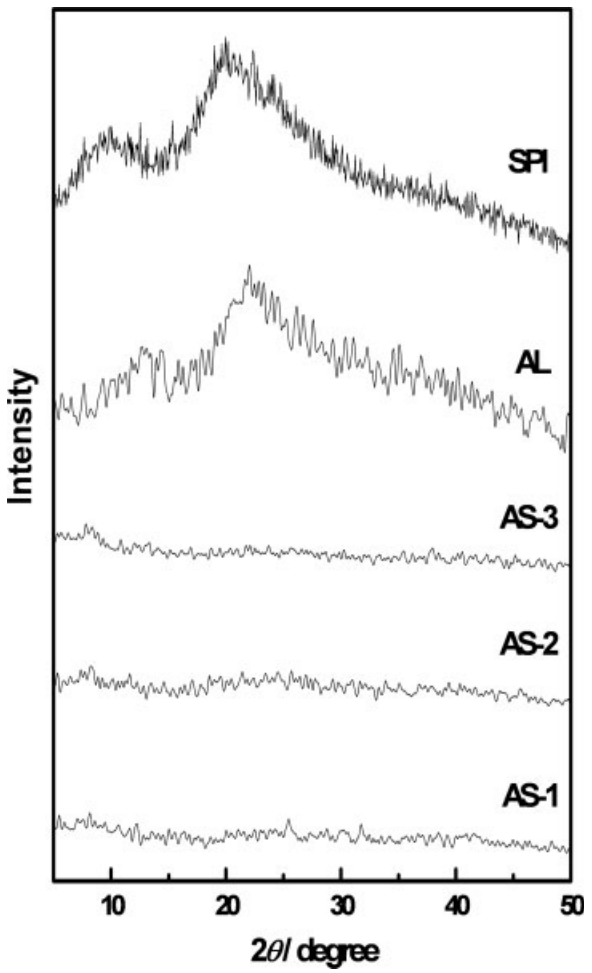

Figure 2 XRD patterns of the complex microspheres based on SPI and AL as well as pure SPI powder and AL microsphere.

namely the complex microspheres were wholly amorphous. It suggested that there was high miscibility between SPI and AL components in three complex microspheres.

Morphologies of Complex Microspheres

Figure 3 shows the SEM images of free surfaces and cross-sections of freeze-dried complex microspheres and pure AL microsphere, and the difference of miscibility between AL and SPI components can be deduced by comparing the structural changes. When compared with the smooth surface with some small pleats for pure AL microsphere in Figure 3(A), the SA-1 microsphere containing 25 wt % SPI showed a rougher surface in Figure 3(B) because introducing SPI destroyed the original structure of AL component. However, when the SPI content increased up to 50 wt %, a smooth surface was observed for the SA-2 microsphere [Fig. 3(C)], indicating the highest miscibility between SPI and AL components under such weight ratio. As the SPI became dominant component, a coarse surface reappeared for the SA-3 microsphere containing 75 wt % SPI, shown as an alveolate structure in Figure 3(D).

The cross-sections of the freeze-dried microspheres also changed with increasing SPI content. In a whole, all the microspheres showed an internal hollow structure, which was filled with water before freeze-drying. However, after adding 25 wt % SPI, the internal pore size of SA-1 microsphere in Figure 3(F) increased while the coarse wall with some cracks can be identified, which was different from the uniform and interfingering porous structure of pure AL microsphere in Figure 3(E). However, the porous structure was not observed for the SA-2

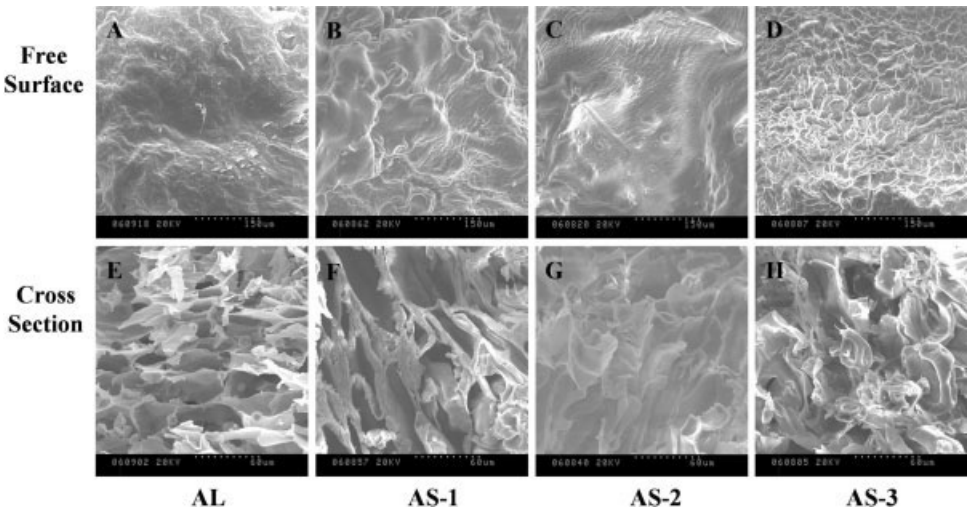

Figure 3 SEM images of the surfaces (A, B, C, D) and cross section (E, F, G, H) for the complex microspheres of SA-1 (B, F), SA-2 (C, G) and SA-3 (D, H) as well as the pure AL microsphere (A, E).

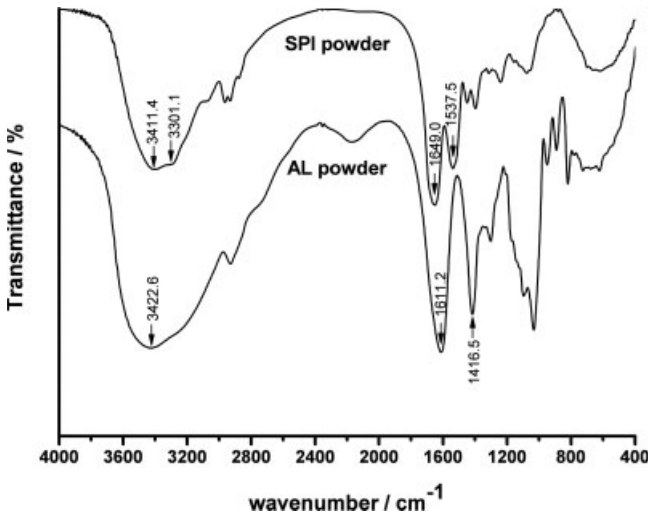

Figure 4 FTIR spectra of AL and SPI powders.

microsphere in Figure 3(G), which was replaced by the lamellae-stacking structure. The smooth lamellae indicated the highest miscibility between components, which is in agreement with the surface observation. Thereafter, when the SPI content increased up to 75 wt %, tens micrometers of aggregates attached onto the internal pores.

Interaction between SPI and AL components

Usually, the good miscibility in the blend is driven by the intermolecular hydrogen bond among components. Before discussing the interaction between components, the groups that possibly form hydrogen bonds must be identified. The FTIR spectra of AL and SPI powder were shown in Figure 4. Except for the double peaks at 3411.4 and 3301.1 cm^{-1} assigned to O—H and N—H stretching vibrations, the SPI powder had two characteristic peaks at 1648.8 (amide I) and 1537.5 cm^{-1} (amide II),[5] which can reflect the formation or cleavage of hydrogen bonds. The O—H groups in AL can also anticipate into hydrogen bonding, and its stretching vibration located at 3422.6 cm^{-1}. In addition, the —COOH group can be crosslinked with Ca^{2+}, which constructs the structure of microsphere and plays a stabilizing role. The —COOH has asymmetrical and symmetrical stretching vibrations located at 1611.2 and 1416.5 cm^{-1}, respectively; the former is stronger and wider while the latter is sharp.

FTIR spectra of the freeze-dried microspheres with different SPI content are shown in Figure 5. After the AL microsphere formed by the Ca^{2+} crosslinking, the absorption of O—H stretching vibration at 3422.6 cm^{-1} of pure AL powder was shifted down to 3376.7 cm^{-1}, indicating that there existed stronger hydrogen bonding. Meanwhile, the crosslinking of Ca^{2+} caused two peaks assigned to asymmetrical and symmetrical stretching vibration of —COOH approached each other after ionization (form $—COO^-$), and located at 1608.6 and 1433.9 cm^{-1}. The changes of SPI content in complex microspheres can be observed by the intensity changes of characteristic peak of SPI component, namely the intensities of peaks at 1634.5 and 1544.9 cm^{-1} increased with increasing SPI content. Correspondingly, the peak intensities at 1608.6 and 1433.9 cm^{-1} assigned to AL component decreased. Introducing SPI containing N—H groups resulted in the shift of absorption above 3000 cm^{-1} to high wavenumber for the complex microsphere, shown as the peak position at 3384.9 cm^{-1} in FTIR spectra of SA-1 microsphere. Meanwhile, the C=O stretching vibration assigned to SPI shifted to low wavenumber of 1634.5 cm^{-1} in contrast to SPI power, indicating that the SPI component in SA-1 microsphere participated the formation of hydrogen bonds. When the SPI content increased up to 50 wt % (SA-2 microsphere), the maximum absorption above 3000 cm^{-1} shift down to 3360.2 cm^{-1}. Thereafter, with an unceasing increase of SPI content, the peak position of SA-3 microsphere shifted to high wavenumber of 3370.1 cm^{-1} again. As a result, the absorption of O—H and N—H

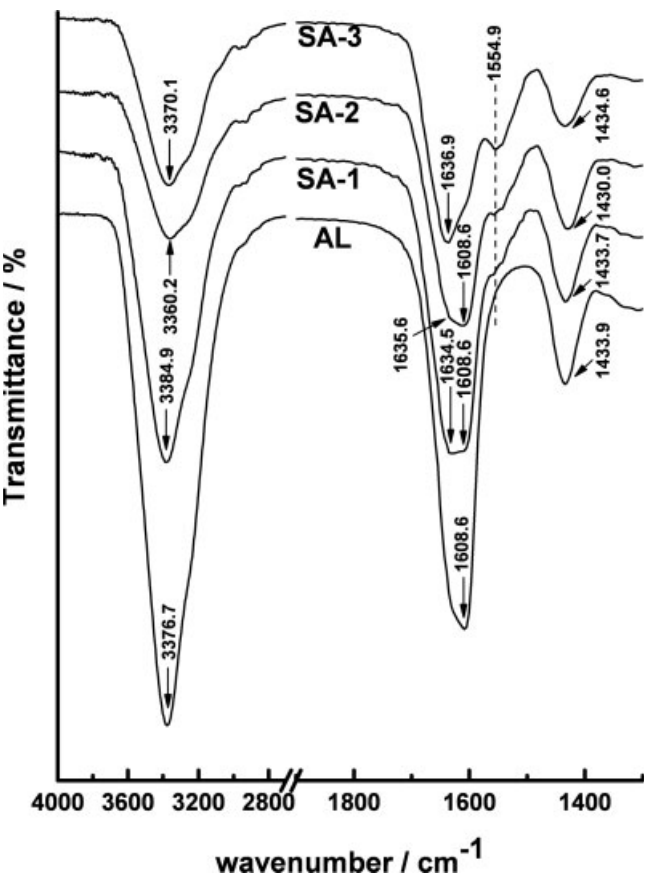

Figure 5 FTIR spectra of the SA complex microspheres and pure AL micrpsphere after freeze-drying.

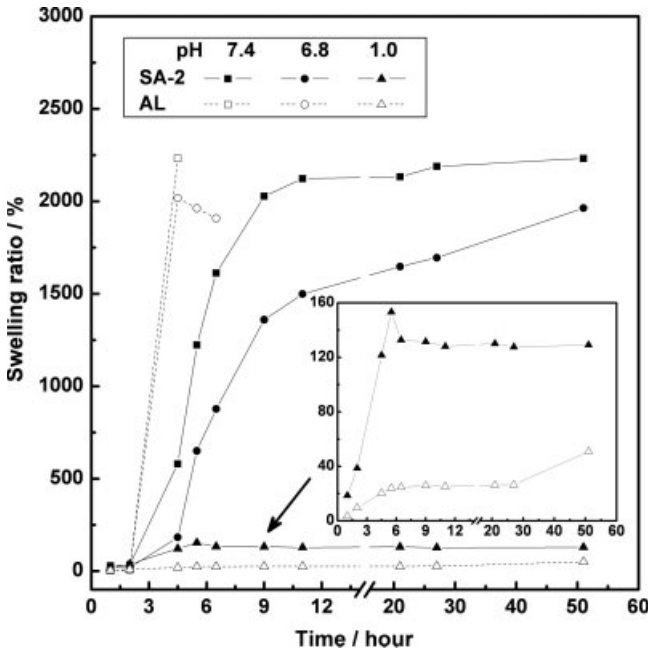

Figure 6 The time dependence of swelling ratio of the SA-2 complex microsphere and pure alginate microsphere (AL) in the buffer solutions with pH 1.0, 6.8 and 7.4.

stretching vibrations of SA-2 microsphere located the lowest wavenumber, suggesting hydrogen bonding most strongly. It might be the origin of the highest miscibility of SA-2 microsphere as well as a uniform surface and cross-section structure shown by the SEM images in Figure 3.

Swelling behaviors of complex microspheres

Figure 6 shows the swelling behavior of pure AL microspheres (AL) and the selected AL/SPI complex microspheres (SA-2) in the HCl solution of pH 1.0 and phosphate buffer solutions of pH 6.8 and 7.4, which correspond to the pH values of stomach, intestine, and colon respectively. Because of the pH sensitivity of the linkage between Ca^{2+} and COO^{-} on AL molecules, the AL microsphere and complex microsphere containing AL component had a pH-dependent swelling character. The AL microsphere did not swell at pH 1.0, but rapidly swelled at pH 6.8 and 7.4 and became sol state in 5 h. However, after adding SPI component, the SA-2 microsphere showed a tendency of slow swelling at pH 6.8 and 7.4, namely the SA-2 complex microsphere gradually swelled and reached the equilibrium at about 11 h. The maximum swelling ratio of SA-2 microsphere can reach $\sim$ 2000% in the solutions of pH 6.8 and 7.4 at 60 h. The higher stability of the SA-2 microsphere at pH 6.8 and 7.4 attributed to the filling of SPI component and the hydrogen bonding between components. However, when the Ca^{2+} crosslinking can be stable at pH 1.0, the addition of SPI resulted in partly damage of AL structure crosslinked by Ca^{2+}. Even though the hydrogen bonds formed between SPI and AL components, the swelling ratio slightly increased. When compared with $\sim$ 20% of maximum swelling ratio of AL microsphere, the SA-2 microsphere showed higher swelling ratio of $\sim$ 160%. The results of swelling tests forecasted that the AL/SPI complex microspheres can be used to the controlled slow-release of drug in intestine and colon in spite of the slight higher swelling ratio in pH 1.0 of stomach, which is superior to the rapid cleavage of pure AL microsphere in pH conditions of intestine and colon.

Drug release from complex microspheres

As reported,[30] the physical state may be changed after encapsulation. Figure 7 shows the XRD patterns of pure theophylline and theophylline loaded SA-2 microsphere. Obviously, the crystalline theophylline has been transformed into the amorphous state by the process of encapsulation. Such conversion benefited the dissolution of drug in aqueous media.[31] Figure 8 shows the drug-release behavior of theophylline-loaded SA-2 microspheres in the HCl solution of pH 1.0 (stomach) and phosphate buffer solutions of pH 6.8 (intestine) and 7.4 (colon). On the basis of the effects of pH on the swelling behavior, the pH values show a insignificant effect on drug release of theophylline-loaded SA-2 microsphere. The maximum content of theophylline released from SA-2 microsphere reached 74.6% at 10 h in buffer solution of pH 7.4 and 76.2% at 10 h in buffer solution of pH 6.8. Under the conditions of low swelling ratio (the solution of pH 1.0), the released content of theophyl-

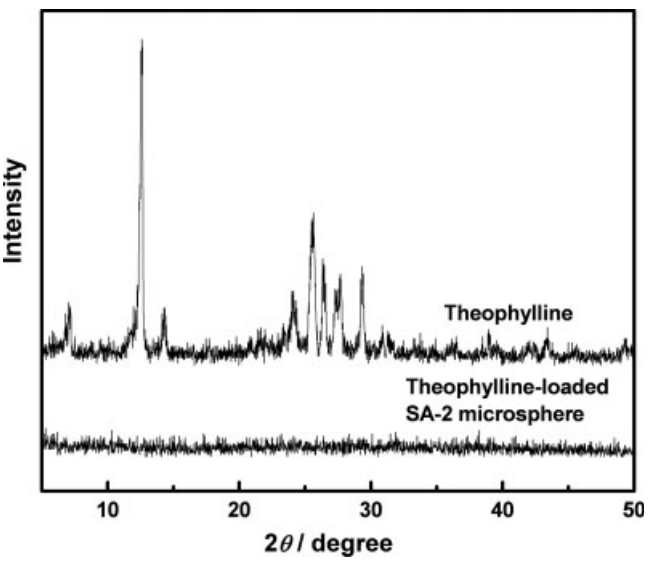

Figure 7 XRD of theophylline and theophylline-loaded SA-2 microsphere.

line can reach a value of 40.1% at ~ 10 h. Thus, it can be seen that the drug tended to be released in pH conditions of intestine and colon.

Although the maximum release content in pH 1.0 is relatively higher, limiting the release time in 4 h lowered release content down to only 15.6%. Thereupon, the experiment of drug release under pH-gradient was carried out, which simulated the whole process of drug in human body. It started from the solution of pH 1.0 (stomach) and then placed into the solution of pH 6.8 (intestine) followed by immersing into the solution of pH 7.4 (colon). Figure 9 shows the drug-release process of theophylline-loaded SA-2 microsphere in designed pH-gradient conditions, and the arrows in Figure 9 point out the starting points of different pH values. The results of drug release in pH-gradient showed a similar character to those in simple pH value. After a small amount of drug was released in the solution of pH 1.0, the drug still encapsulated by the SA-2 microsphere can be rapidly released with the rapid swelling in the solutions of pH 6.8 and 7.4. At last, the released content of drug can reach higher than 75%. Meanwhile, the percentage of the released content in the pH conditions of intestine and colon was about 86.1% for the delay of 2 h and about 80.0% for the delay of 4 h in the solution of pH 1.0, suggesting that the AL/SPI complex microspheres have a function of controlled release in the sites of intestine and colon. On the basis of the results of drug release *in vitro*, the process of drug release *in vivo* can be described as follows. When entering the stomach, the drug-loaded SA microspheres slowly swell to release about 5–15% drug depending on the lag time (2 and 4 h). Subsequently, the drug-loaded SA microspheres enter into the intestine site and swell rapidly to release more drugs. After settling in the intestine for 2–4 h, the drug-loaded SA microspheres reach the colon and keep on the rapid release of drugs with further rapid swelling.

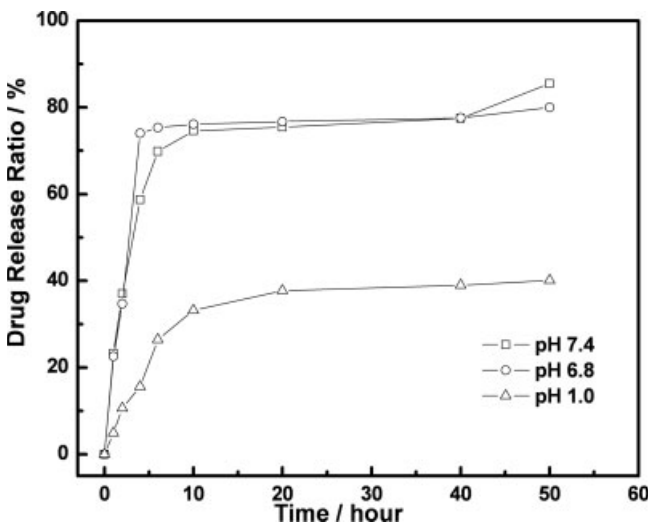

Figure 8 The time dependence of drug release ratio of the SA-2 complex microsphere in solutions with pH 1.0, 6.8 and 7.4.

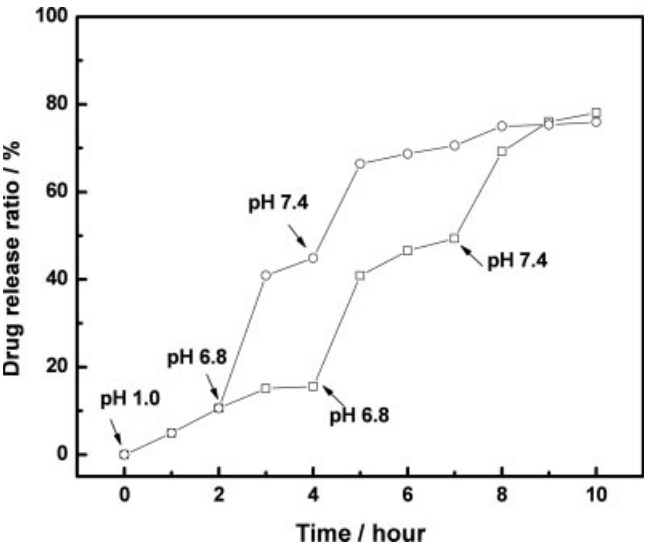

Figure 9 The time dependence of drug release ratio of the SA-2 complex microsphere in the solutions with pH gradient.

CONCLUSION

In summary, a potential drug-delivery system based on AL and SPI is developed, which combines the pH sensitivity of AL with the bioactivity of SPI. This system shows a form of microsphere and is stabilized by the crosslinking of Ca^{2+}. The best miscible microsphere contains the weight ratio of SPI and AL by 1 : 1, and presents a uniform and smooth structure. Furthermore, the strong interaction among components, such as hydrogen bonding, is the main driving force to promoting the miscibility. Different from the rapid cleavage of pure AL microsphere in the conditions of pH 6.8 and 7.4, the complex microspheres showed a slow swelling behavior in spite of relatively higher swelling ratio in the condition of pH 1.0. On the basis of the pH-dependent swelling behaviors, the complex microspheres can be used as the controlled-release of drugs. Such complex microspheres are suitable to facilitate the prompt release of drugs in the sites of intestine (pH 6.8) and colon (pH 7.4).

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