# Structure and Control Release of Chitosan/Carboxymethyl Cellulose Microcapsules

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**ABSTRACT:** Microcapsules of chitosan/sodium carboxymethyl cellulose (NaCMC) were successfully prepared using a novel method of emulation phase separation. Their structure and morphology were characterized by infrared spectroscopy (IR), scanning electron microscopy (SEM), and X-ray diffraction. Bovine serum albumin (BSA) was encapsulated in the microcapsules to test their release behavior. The swelling behavior, encapsulation efficiency, and release behavior of the microcapsules with different chitosan contents and pH conditions were investigated. The results indicated that the microcapsules have a high encapsulation efficiency (75%) and a suitable size (20–50 $\mu$m). The BSA in the microcapsules was speedily released at pH 7.2, namely, in intestinal fluid. The BSA release was reduced with increase of the chitosan content from 17 to 38% in the microcapsules. Acid-treated microcapsules have a compact structure, owing to a strong electrostatic interaction caused by —$NH_2$ groups of chitosan and —COOH groups of CMC, and the encapsulated BSA was hardly released at pH 1.0, namely, in gastric juice. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 82: 584–592, 2001

**Key words:** chitosan; carboxymethyl cellulose; microcapsule; bovine serum albumin; control release

## INTRODUCTION

Recently, microcapsules or microspheres based on polymers have attracted much attention as systems of inclusion, for delivering and target-carrying in food, medicine, and pesticides and in commodity industries and for environmental protection. New controlled drug-delivery systems in response to changes in environmental conditions, such as temperature,[1,2] pH,[3–5], light (ultraviolet[6] or visible[7]), electric field,[8,9] and certain chemicals[10] have been explored. There is considerable interest in developing controlled or sustained drug-delivering systems using biopolymers, due to their nontoxicity, biodegradability, and biocompatibility. Chitosan is poly-$\beta$(1→4)-D-glucopyranosamine composed of glucosamine and a *N*-acetyl glucosamine unit[11] and the second most plentiful natural biopolymer with mucoadhesivity,[12] biocompatibility, and nontoxicity.[13] In addition, chitosan has the special quality of gelling upon contact with anions, allowing the formation of beads under very mild conditions.[14] The chitosan bead is pH-dependent on the swelling behavior and is appropriate for the delivery of drugs in the gastric cavity. Moreover, agents with a chitosan matrix exist as a gel form at a low pH

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value to retard the diffusion of the bioactive agent.[15] Chitosan/poly(ethylene glycol)–alginate microcapsules have been used for the encapsulation of albumin and hirudin.[16] The acid-treated microcapsules released almost all the hirudin into a Tris–HCl buffer (pH 7.4) within 6 h, due to the hydrolysis of alginate. Nanoparticles prepared from chitosan–poly(ethylene oxide) as protein carriers have an entrapment efficiency up to 80%.[17]

Plant cellulose, an environmentally friendly material, is the richest natural polymer on earth, where hundred of millions of tons of cellulose occur per year.[18] Cellulose has been reevaluated as a functional material because of its unique reactivation and molecular characteristics and is nontoxic, biodegradable, biocompatible, hydrophilic, chiral, and semirigid.[19] The blends of cellulose or carboxyl-methylated (CM) chitosan with alginate were investigated in our laboratory, indicating that a strong interaction was caused by the hydrogen bonding that occurred between the cellulose and alginic acid molecules or the electrostatic force between the —COOH groups of alginic acid and the —$NH_2$ groups of CM–chitosan.[20,21] Water-soluble carboxymethyl cellulose (CMC) can also provide —COOH groups to blend easily with chitosan.

In this work, we attempted to prepare chitosan/CMC microcapsules by an emulsion phase-separation method and studied the effects of the components and structure on the release. IR and scanning electron microscopy (SEM) were used to characterize the structure and morphology of the microcapsules. It is well known that proteins are quickly denatured and degraded in the hostile environment of the stomach. In this case, protein encapsulated in a shell can be protected from biodegradation. Hence, bovine serum albumin (BSA) was encapsulated in the capsules to test the release. The swelling behavior, crystallinity, and release behavior of the microcapsules with and without BSA in different pH conditions were examined and are discussed.

## EXPERIMENTAL

### Materials

Chitosan (supplied by the Yuhuan Ocean Biochemistry Co. Ltd., Zhejiang Province, China) with 87% deacetylation was used after screening and micronizing with a 60-mesh sieve. Its viscosity-average molecular weight $M_\eta$ was determined to be $8.4 \times 10^5$ using viscometry according to the Mark–Houwink equation[22]: $[\eta] = 1.424 \times 10^{-3} M^{0.96}$ (mL $g^{-1}$). Sodium carboxymethyl cellulose (NaCMC) was purchased from the Shanghai Chemical Reagent Co. (Shanghai, China) and was micronized (>80 mesh) before use. The $M_\eta$ of NaCMC was measured in cadoxen at 25°C and was calculated to be $1.61 \times 10^5$ according to $[\eta] = 3.34 \times 10^{-2} M^{0.73}$ (mL $g^{-1}$).[23] Span-20 was obtained from the Shanghai Chemical Reagent Co. BSA was of V grade and purchased from the German B.M. Co. (German). All other materials were of analytical grade.

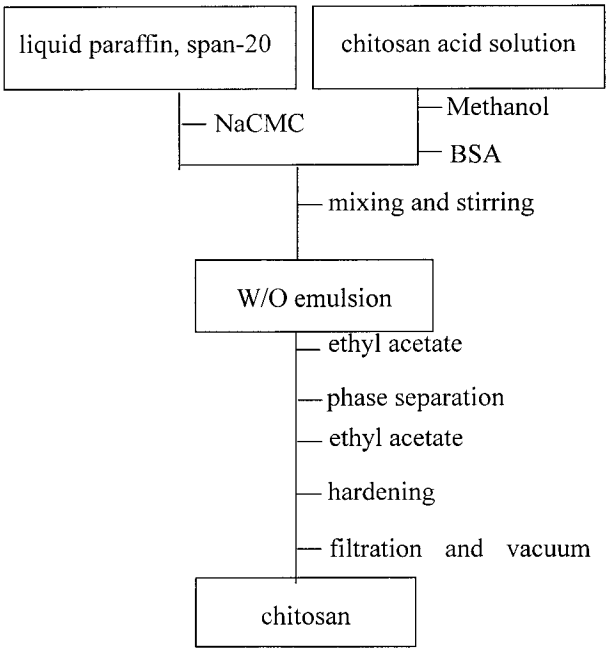
**Table I Compatibility Between Chitosan Acidic Solution and Organic Solvents**

| Chitosan Solution In | Methanol | Absolute Ethanol | Acetone |
|---|---|---|---|
| Hydrochloric acid | + | ++ | +++ |
| Acetic acid | − | − | + |
| Ascorbic acid | − | + | +++ |
| Citric acid | − | ++ | ++ |

(−) Soluble; (+) insoluble; (++ and +++) larger white precipitate.

### Preparation of Chitosan/NaCMC Microcapsules

To clarify the compatibility of chitosan in an acidic solution with an organic solvent, a 1.67% (w/v) chitosan acid solution was prepared by dissolving 0.25 g of the chitosan in 15 mL of a 2% acidic aqueous solution, in which HCl, acetic acid, ascorbic acid, or citric acid was used as the acid source. An organic solvent such as methanol, absolute ethanol, or acetone was added to each chitosan acidic solution and a precipitation phenomenon was observed. The methanol, ethanol, and acetone possess a hydrophilic property, which is immiscible with hydrophobic liquid paraffin. If the organic solvents were separately added to liquid paraffin, spherical drops of the solvent can be formed by an interfacial phenomenon. However, chitosan does not dissolve in an organic solvent, but dissolves only in aqueous organic acids. Thus, the compatibility between organic solvents and the chitosan acidic solution was determined by the eye. Table I shows the compatibility of four acid solutions of chitosan with three organic solvents. Apparently, the compatibility of methanol and chitosan solutions is good, whether in acetic




**Scheme 1** Preparation of chitosan microcapsules by emulsion separation method.

acid, ascorbic acid, or citric acid. Therefore, methanol and acetic acid were selected to be used here.

Chitosan microcapsules were prepared by the emulation phase-separation method at room temperature. Ten grams of NaCMC powder was previously suspended in 100 mL of liquid paraffin containing 0.7% of span-20. Twenty milliliters of acetic acid with different concentrations of chitosan containing 10 mL of methanol and 1.0 g of BSA were added to the liquid paraffin system to form a water-in-oil (W/O) emulsion, stirring at a speed of 600 rpm for 1 h to settle the particles of emulsion. Thereafter, 50 mL ethyl acetate was added in drops to the above system, and the phase separation occurred at the interface of the W/O emulsion to form hard-shelled microcapsules. The microcapsules were resuspended in 50 mL ethyl acetate overnight to remove the residual oil and then continued to harden in ethyl acetate. The microcapsules obtained were filtrated, air-dried at room temperature, and then vacuum-dried. The overall process is outlined in Scheme 1. By changing the content of chitosan (0.2, 0.4, 0.6 g) in 20 mL acetic acid, a series of the encapsulation microcapsules were prepared and coded as CSCM-B1, CSCM-B2, and CSCM-B3, and the microcapsules without BSA, coded as CSCM-1, CSCM-2, and CSCM-3. The dry CSCM-1 microcapsules were immersed both in 0.1*M* HCl (pH 1.0) and in Tris–HCl buffer (pH 7.2) at room temperature for 24 h, then dried to obtain acid-treated and buffer-treated microcapsules coded as CSCM-1a and CSCM-1b, respectively.

### Characterization

IR spectra of chitosan, CMC, CSCM-1, CSCM-1a, and CSCM-1b were recorded with a Fourier transform infrared spectrophotometer (FTIR, Nicolet 170SX). The crystallinity of the microcapsules was measured with an X-ray diffractometer (Rigaku Dmax-$\gamma$A). The X-ray diffraction patterns with CuK$\alpha$ at 40 kV and 50 mA were recorded in the region of $2\theta = 8-60°$. The surface of the microcapsules was coated with gold in a 0.1$\tau$ vacuum degree, then observed and photographed on a Hitachi S-570 scanning electron microscope. The size of the microcapsules was calculated according to the magnification of the graphs. Every kind of particle was measured three times to obtain the average size.

### Swelling Test

The preweighted dry microcapsules (100 mg) not containing BSA (CSCM-1) were immersed both in 0.1*M* HCl (pH 1.0) and in Tris–HCl buffer (pH 7.2) for 24 h at room temperature until a swollen equilibrium was reached. The swollen samples were collected by a centrifuge, and the wet weight of the swollen microcapsules was determined by first blotting the particles with filter paper to remove the adsorbed water on the surface and then weighing immediately on an electronic balance.[11] The degree of swelling (*SW*) was calculated by

$$SW\ (\text{wt}\ \%) = [(w - w_0)/w_0] \times 100\%$$

where $w$ and $w_0$ are the weights of the microcapsules at the equilibrium swelling state and the dry state, respectively.

### BSA Encapsulation Efficiency of the Microcapsules

Usually, for the microcapsules prepared from the oil phase, it is impossible to measure the amount of free BSA in the supernatant. To calculate the BSA encapsulation efficiency (*AE*), the amount of BSA released in the solution after 48 h can be presumed as the loading amount of BSA, which was determined by UV spectrophotometry at $\lambda = 279$ nm until an unchangeable value. The BSA

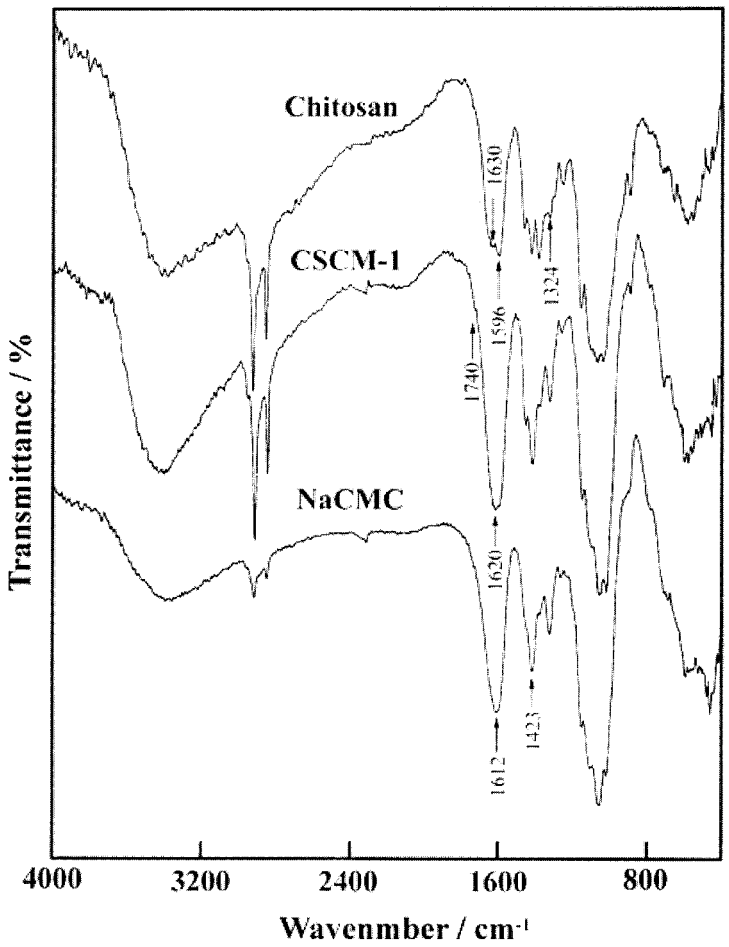



**Figure 1** IR spectra of chitosan, CSCM-1, and NaCMC.

encapsulation efficiency (*AE*) of the microcapsules was calculated as following equation:

$$AE = (\text{loading amount BSA/total amount BSA}) \times 100\%$$

where the total amount BSA is added to original amount of BSA for the microcapsules.

### BSA *in Vitro* Release Test

The release of encapsulated BSA was carried out in 0.1*M* HCl (pH 1.0) and Tris–HCl buffer (pH 7.2) at 37°C. The sample solutions at appropriate intervals was withdrawn and assayed using Lowry's method[24] for protein estimation. An equal volume of the same dissolution medium was added to maintain a constant volume. The release assay was reproduced twice to obtain the average value. The BSA release amounts were plotted as the cumulative amount and percentage content in the dissolution medium against the release time.

## RESULTS AND DISCUSSION

### Structure and Morphology of Microcapsules

The IR spectra of chitosan, NaCMC, and CSCM-1 are shown in Figure 1. The characteristic peak of the amino groups was at 1596 $cm^{-1}$, and amide I and amide III bands at 1630 and 1324 $cm^{-1}$ appeared in the chitosan.[25,26] In the spectrum of NaCMC, two strong peaks at 1612 and 1423 $cm^{-1}$ were observed due to the asymmetrical and symmetrical stretching of $COO^-$ groups. The amide I band at 1630 $cm^{-1}$ in CSCM-1 changed to 1620 $cm^{-1}$, due to the association between chitosan and NaCMC. A weak shoulder peak at 1740 $cm^{-1}$, which reflects an interaction between the —COOH groups of CMC and the —$NH_2$ groups of

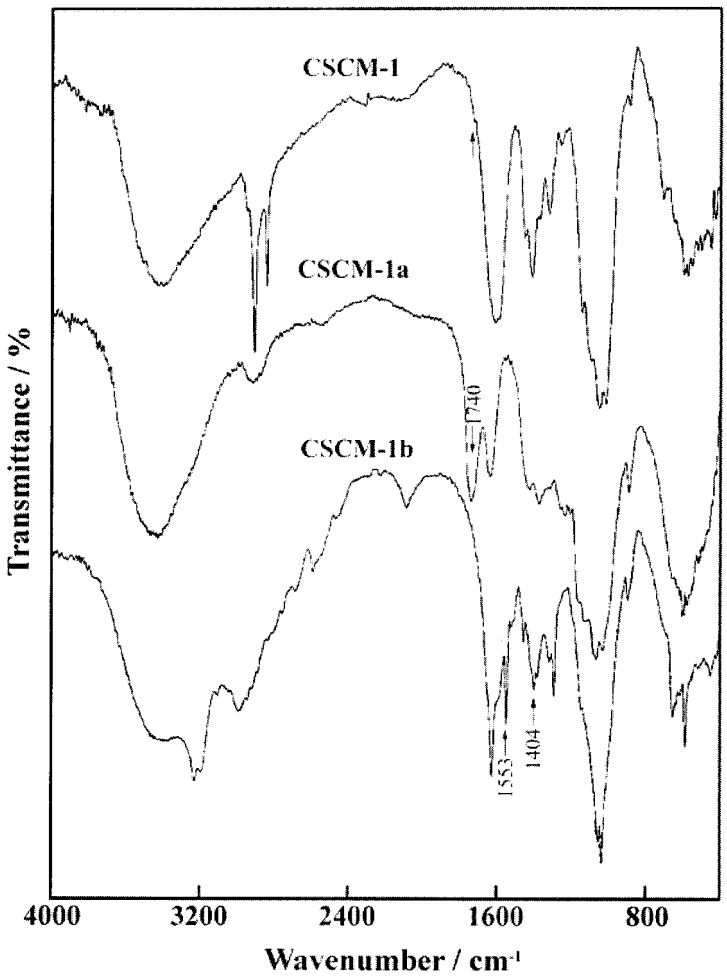



**Figure 2** IR spectra of the CSCM-1, CSCM-1a, and CSCM-1b microcapsules.

chitosan, appeared in the IR spectrum of CSCM-1. In addition, the —OH stretching vibration bands of the CSCM-1 microcapsules was narrowed and shifted to a higher wavenumber compared with the NaCMC and chitosan (at 3400 $cm^{-1}$), suggesting that the original intermolecular hydrogen bonding in NaCMC and chitosan was broken in the microcapsules, due to the blend of two polymers. The results indicated that the microcapsules were composed of chitosan and NaCMC.

Figure 2 shows the IR spectra of acid-treated CSCM-1a microcapsules and buffer-treated CSCM-1b microcapsules. It is obvious that, compared with CSCM-1 and CSCM-1b, a new peak at 1740 $cm^{-1}$ appeared in CSCM-1a, which was caused by the electrostatic interaction between the —COOH groups of CMC and the $—NH_2$ groups of chitosan.[21] The peaks at 1553 and 1404 $cm^{-1}$ in CSCM-1b due to the asymmetrical and symmetrical stretching of the $COO^-$ groups appeared, suggesting that the interaction between chitosan and CMC was significantly weakened.

SEM photographs of the microcapsules are shown in Figure 3. The sizes of the prepared microcapsules are all about 40–50 $\mu$m, and CSCM-1 and CSCM-B1 have good sphericity [Fig. 3(a,b)]. The surface of the microcapsules was surrounded with some residual CMC. The size of the CSCM-1 microcapsules was smaller than that of CSCM-B1, which encapsulated BSA. Interestingly, the shape of the CSCM-1b microcapsules was not spherical, and the surface had porous, open channels [Fig. 3(d)]. This may be caused by weakening of the interaction between CMC and chitosan. However, the acid-treated CSCM-1a microcapsules exhibited a smaller sphere size of 20 $\mu$m with a compact structure, which was caused by shrinking after being treated in 0.1*M* HCl. As shown in CSCM-1a [Fig. 3(f)], the surface mor-

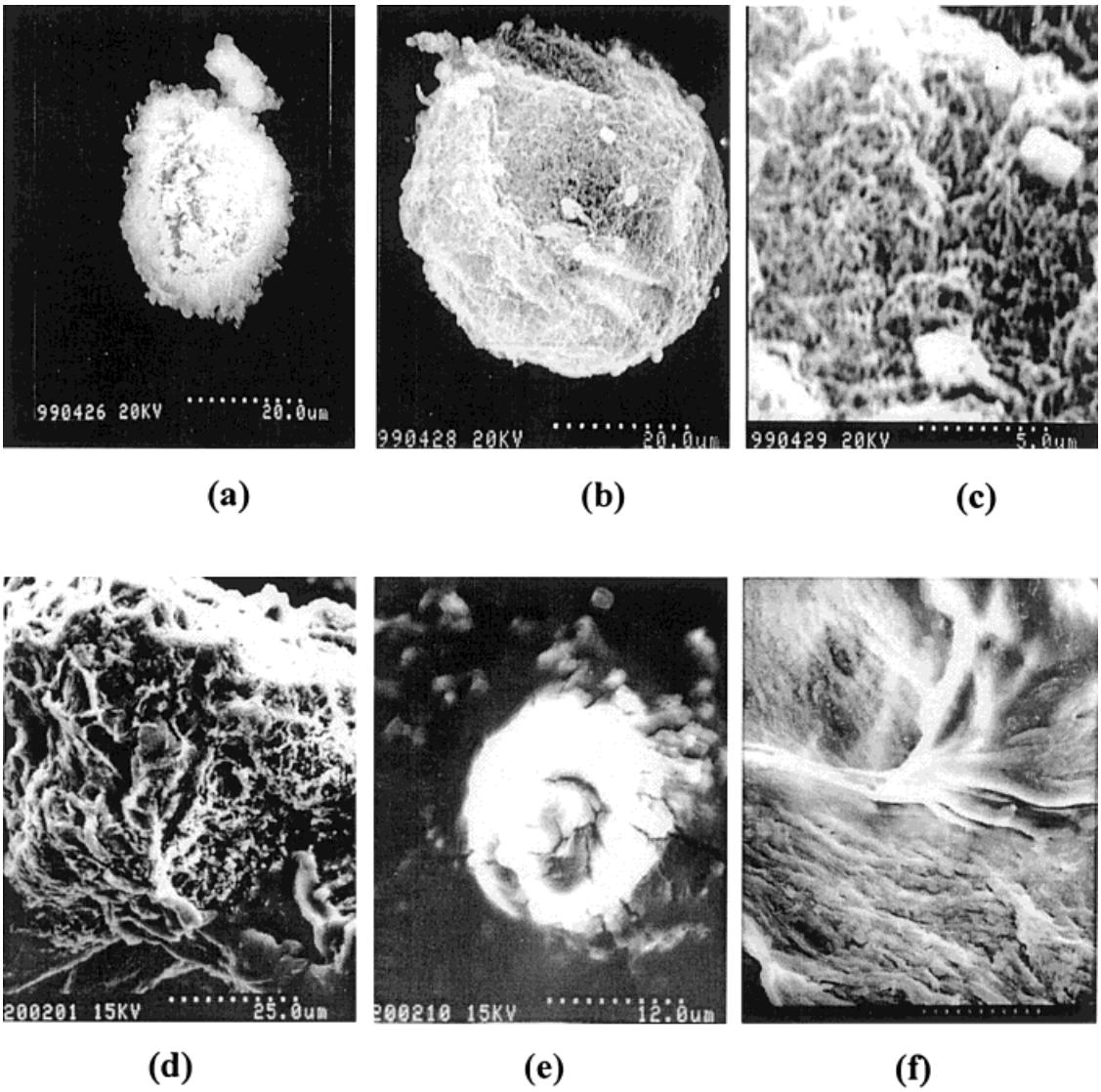



**Figure 3** SEM of the (a) CSCM-1, (b,c) CSCM-B1, (d) CSCM-1b, and (e,f) CSCM-1a microcapsules.

phology was denser and smoother than was that of CSCM-1, CSCM-B1, and CSCM-1b, suggesting that a strong interaction between chitosan and CMC in the microcapsules occurred in the acidic condition. This is in good agreement with the IR results.

Figure 4 shows the X-ray diffraction patterns of CSCM-1 and the microcapsules treated in 0.1*M* HCl (pH 1.0) and in Tris–HCl buffers (pH 7.2). In view of the X-ray diffraction patterns, the crystallinity of CSCM-1a was higher than that of the others. The relatively low crystallinity of CSCM-1b indicated a decrease in the intermolecular interaction, which caused the release process to quicken.

### Effect of Chitosan Concentration on Size and Release Behavior

Figure 5 shows the effect of chitosan concentration on the particle size of the microcapsules. Apparently, the particle size decreased with increase of the chitosan concentration. This can be explained by that chitosan and CMC are two oppositely charged polyelectrolytes, and the microcapsule formed an insoluble complex caused by the hydrogen bonding of two polymers. Therefore, the intermolecular interaction of the NaCMC and chitosan increased with increase of the chitosan from 17 to 38% in the capsules, resulting in shrinkage of the particle size.

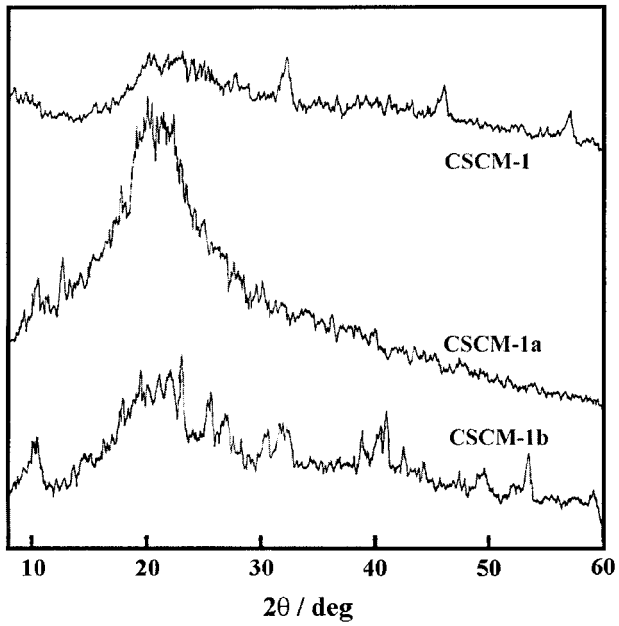



**Figure 4** X-ray diffraction patterns of CSCM-1a and CSCM-1b microcapsules treated with 0.1*M* HCl (pH 1.0) and Tris–HCl buffers (pH 7.2), respectively, and CSCM-1.

The values of the BSA encapsulation efficiency (*AE*) of the CSCM-B1, CSCM-B2, and CSCM-B3 microcapsules were calculated to be around 75%, from reproducible results obtained three times. So, the chitosan concentration hardly affected the entrapment of BSA. Compared with the literature, the microcapsules have a relatively high loading capacity. This may be related to the isoelectric point of BSA.

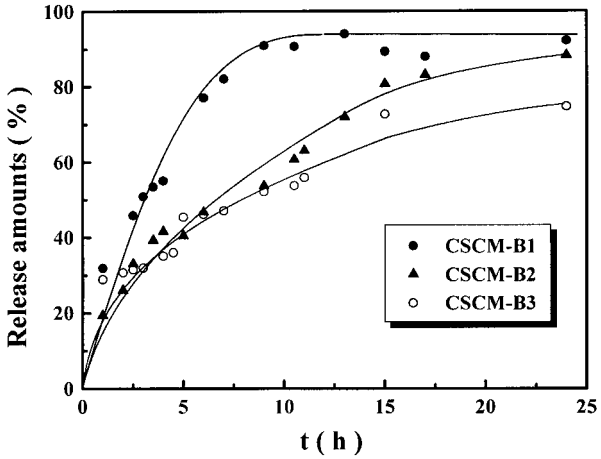



**Figure 6** Effect of chitosan concentration on BSA release from the microcapsules in Tris–HCl buffer, at pH 7.2 and 37°C.

Figure 6 shows the effects of the chitosan content on the release behavior of the microcapsules in the Tris–HCl buffer (pH 7.2) at 37°C, which indicated that in the first 5 h CSCM-B1 released nearly 60% BSA; however, CSCM-B2 and CSCM-B3 only released 35% BSA. After 1 day, CSCM-B1 released 95% BSA, but CSCM-B2 and CSCM-B3 only released 80 and 65% BSA, respectively. In other words, the BSA in the microcapsules containing more chitosan such as 38% was more slowly released because of a relatively compact surface structure.

### Effect of pH on Release

Figure 7 shows the BSA *in vitro* release behavior of the acid-treated and buffer-treated CSCM-1a

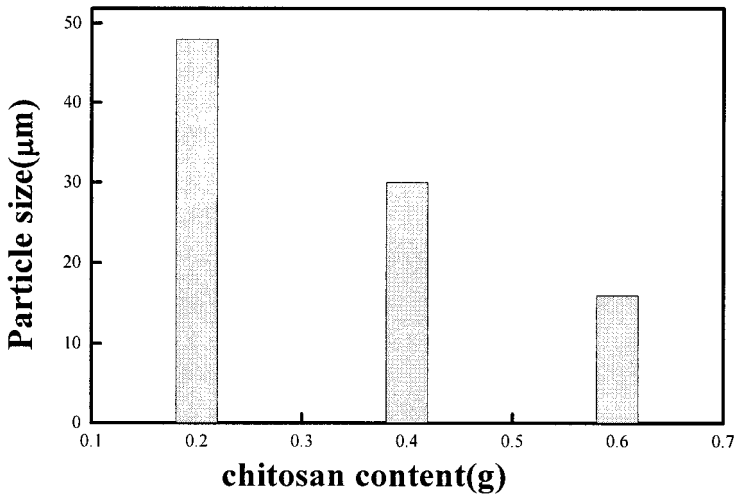



**Figure 5** Effect of chitosan concentration on particle size.

and CSCM-1b microcapsules. The results indicate that the release of BSA depends obviously on the pH. Compared with the release curve in pH 7.2, a few BSA in the CSCM-1a was released at pH 1.0, owing to the stronger interaction between CMC and chitosan to form a dense surface membrane. In the first 4 h, only 20% BSA was released from CSCM-1a, while in CSCM-1b, it was more than 50%. The fast release of BSA in pH 7.2 is due to the easy penetration of the buffers into porous microcapsules, quickly inducing the swelling of the polymer. More than 90% of BSA was released from CSCM-1b in 24 h. Therefore, the microcapsule is pH-sensitive and has a potential application in controlled release such as in intestinal fluid or gastric juice.

### Effect of pH on Swelling of Microcapsules

The pH dependence of the swelling of the microcapsules is shown in Figure 8. The microcapsules in 0.1*M* HCl (pH 1.0) had a lower percentage swelling value, compared to that at pH 7.2. The swelling ability of the microcapsules under an acid environment was weakened. This can be explained in that, in a low pH condition, the amino groups from chitosan were protonated and the electrostatic interaction of carboxyl groups of CMC with the amine groups of chitosan was strengthened, resulting in a dense structure. The conclusion was supported by the SEM result in Figure 3.

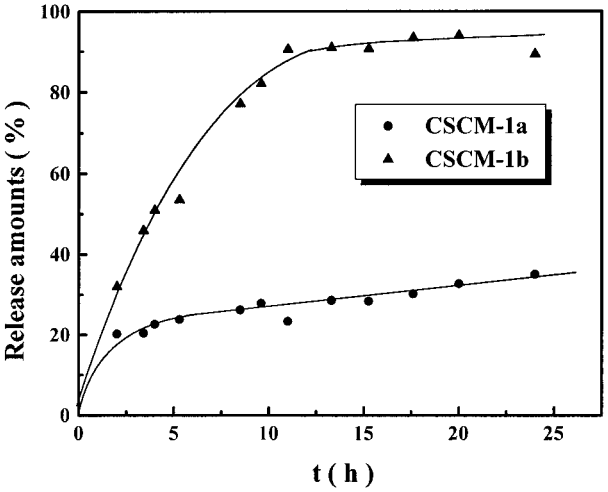



**Figure 7** Time ($t$) dependencies of BSA release from microcapsules being treated in 0.1*M* HCl (pH 1.0) and Tris–HCl buffers (pH 7.2).

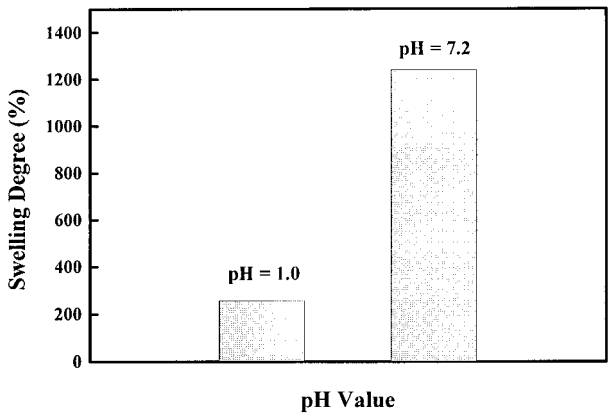



**Figure 8** Swelling degree of the microcapsules in different pH conditions.

## CONCLUSIONS

The microcapsule spheres encapsulating BSA were preliminary prepared from chitosan and NaCMC using a novel method of emulsion phase separation. The BSA encapsulation efficiency was around 75%. The size of the microcapsules ranged from 20 to 50 $\mu$m and changed with the chitosan content and acid treatment. The BSA in the microcapsules was speedily released in the Tris–HCl buffer (pH 7.2), namely, in intestinal fluid, and the release decreased with increase of the chitosan content from 17 to 38% in the microcapsules. The dry microcapsules were immersed in 0.1*M* HCl (pH 1.0) at room temperature for 24 h to obtain acid-treated capsules, which have a compact structure, owing to a strong electrostatic interaction caused by —COOH groups of CMC and —$NH_2$ groups of chitosan. The encapsulated BSA in the acid-treated microcapsules was hardly released at pH 1.0, namely, in gastric juice. This study will be continued to provide the exact outcome of the released drug.

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