

# Fabrication and property of chitosan film carrying ethyl cellulose microspheres

Pujiang Shi, Yubao Li <sup>\*</sup>, Li Zhang

*The Research Center for Nano biomaterials, Analytical and Testing Center, Sichuan University, Chengdu 610064, China*

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## Abstract

Chitosan (CS) film carrying ethyl cellulose (EC) microspheres (comatrix) is reported in this paper. The properties including interaction, shape change under different condition and its mechanical variety in different ratio of CS to EC microspheres were analyzed by using scanning electron microscope (SEM), X-ray diffraction (XRD), infrared spectra (IR), differential scanning calorimetry (DSC) and mechanical testing. The results indicated that the round EC microspheres dispersed in the CS film, and there was interaction between EC and CS matrix. The comatrix may offer an appropriate property for degradation and drug releasing.

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**Keywords:** Chitosan; Film; Ethyl cellulose; Microsphere

## 1. Introduction

Drug delivery system is a field attracting much attention. Many polymers have been used as drug carriers, such as synthetic compounds (polyacrylates, -methacrylate and methacrylestes), semi-synthetic compound (methyl-, ethyl-, hydroxycellulose) and natural compounds (protein, chitosan and alginate). It is reported that ethyl cellulose microcapsules can slow drug releasing rate (Gunder, Lippold, & Lippold, 1995). The ethyl cellulose dispersion with different plasticizers as diffusion pellets coating can obviously give them different drug releasing rate (Frohoff-Hulsmann, Lippold, & McGinity, 1998). When dimenhydrinate is in ethyl cellulose matrix, releasing rate of dimenhydrinate fits to zero-order kinetics; the more ethyl cellulose was in the matrix, the slower the drug releasing rate became (Desai, Alexander, & Riga, 2006). Ethyl cellulose microspheres carrying diclofenac sodium are made by using stabilizers, such as PVA, alginate, pectin and gelatin, which have excellent drug releasing property (Jani & Gohel,

1997). When they are blended by using different plasticizers, CS and ethyl cellulose always have phase separation, however, the interaction between them has been detected (He, Du, & Fan, 2006). CS microspheres in PLG film shows unusual sustained releasing rate, compared with microspheres (Blanco, Gomez, Olmo, Muniz, & Teijon, 2000). The cytarabine releasing rate of albumin microspheres carried by film is slow in vivo and in vitro; when the comatrix is implanted in the rat, no repulsion reaction takes place (Gomez et al., 2004). In addition, a silica film containing polystyrene microspheres is made by dip coating method to form an inorganic–organic thin film. (Adachi, Suzuki, Kashiwagi, Isobe, & Senna, 1998).

EC is an infirmly polar and water-insoluble polymer. The main chain of EC is given by anhydroglucose units linked by 1,4- $\beta$ -glucodidic bonds. Generally, the OH functional groups are available for ester and ether reactions (Fig. 1a). EC is widely used as coating film of pharmaceutical purpose (Hyppola, Husson, & Sundholm, 1996). On the other hand, chitosan is an N-deacetylation product of chitin and a cationic polysaccharide under acidic conditions. It consists of glucosamine and N-acetylglucosamine units linked through 1–4 glycosidic bonds (Fig. 1b). In

<sup>\*</sup> Corresponding author.

E-mail address: [nic7504@scu.edu.cn](mailto:nic7504@scu.edu.cn) (Y. Li).

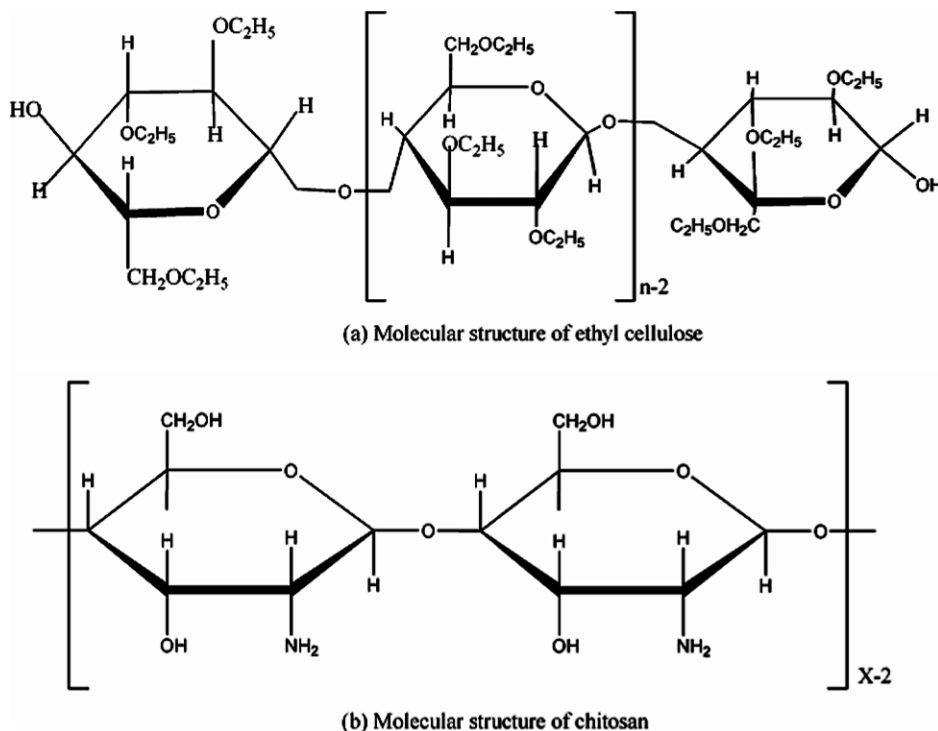


Fig. 1. Molecular structure of ethyl cellulose (a) and chitosan (b).

addition, chitosan is flexible and has a high resistance upon heating, because its intramolecular hydrogen bonds formed between its hydroxyl group and its amino group.

Although the number of articles focusing on microspheres of EC or CS, EC/CS blended film increases quickly, a few researches have been done on film carrying microspheres. In this study, EC is dissolved in acetone–methanol– $\text{CH}_2\text{Cl}_2$  ternary mixture. The EC microspheres, then, are made by solvent remove/solvent evaporation method in CS solution. The comatrix is poured on glass plate to form CS film carrying EC microspheres.

## 2. Materials and methods

### 2.1. Materials

Chitosan was supplied by Haidebei Technological Co. Ltd of Shandong Province, China. Ethyl cellulose was purchased from Niansha Chemical Reagent Company of Kunshan, Jiangsu, China. Methanol, acetone and glacial acetic acid were supplied by Changlian Chemical Reagent Co. Ltd of Chengdu, China.  $\text{CH}_2\text{Cl}_2$  was from Chemical Reagent Factory of Tianjin, China. Other reagents used here were analytical grade.

### 2.2. Microspheres preparation

- (a) Ethyl cellulose microspheres preparation in chitosan solution of various concentrations: EC (0.5 g) was dissolved in ternary mixture (40 ml) consisted of  $\text{CH}_2\text{Cl}_2$ , methanol and acetone. Different chitosan

solution (2%, 4%, 6%, w/v) was prepared by dissolving 2, 4 and 6 g chitosan in 100 ml diluted acetic acid (3% v/v), respectively. The primary O/W emulsion was obtained by injecting 40 ml EC solution in 100 ml chitosan aqueous solution. Emulsification was performed at room temperature under continuous stirring at 1000 rpm for 30 min. The emulsion, then, was put in water incubator for 3 h at 40 °C. After the organic solvent being evaporated, the microspheres were separated by centrifugation, and then they were fully washed and freeze-dried by using distilled water and desiccator.

- (b) Heat treated ethyl cellulose microspheres prepared in chitosan solution: 40 ml above-mentioned ternary solution containing 0.5 g EC was injected in 100 ml 6% CS solution with stirring at 1000 rpm for 30 min. This step was repeated 3 times, respectively. After the three O/W emulsion being formed, they were encircled by water at 40, 70 and 100 °C respectively, until  $\text{CH}_2\text{Cl}_2$ , methanol and acetone evaporated completely. When they were solidified, EC microspheres were separated as above-mentioned methods.

### 2.3. Comatrix fabrication of CS film carrying EC microspheres

CS film carrying EC microspheres was prepared by dripping 40 ml ternary solution containing 0.5 g EC in 100 ml CS solution (6 wt%), then they were stirred at 1000 rpm. Half hour later, the emulsion was maintained at 40 °C.

After the organic solvent being removed, the EC microspheres were formed in CS aqueous solution. The CS film containing EC microspheres was obtained by the following steps: first, the CS solution containing EC microspheres was poured on glass plate, dehydrated at room temperature; second, 1% NaOH solution was added to neutralize the residual acetic acid; last, the comatrix was dehydrated again in vacuum.

#### 2.4. Characterization analyses

The EC microspheres shaped under various condition and the CS film carrying EC microspheres were characterized by SEM (Jeol LTD, Tokyo, Japan). Samples for SEM observation were sputter-coated under argon atmosphere with a thin layer of Au/Pd. The acceleration voltage of SEM was 20 kV for the samples of EC microspheres and the section of the CS/EC film, and 5 kV for the surface of the film. In addition, the thickness of the film was tested by electronic digital outside micrometer (Chengliang Co. Ltd of Chengdu, Chengdu, China). For actual data, five tests were taken for the sample.

Infrared (IR) spectra of ethyl cellulose, chitosan, chitosan–ethyl cellulose (CS–EC) physical mixture and CS film carrying EC microspheres were recorded with a FT/IR spectrophotometer (American Perkin-Elmer Co.). Samples were scanned from 500 to 4000  $\text{cm}^{-1}$ .

DSC analysis was performed by a DSC 200 PC (NET-ZSCH, Germany). Samples (8 mg) were scanned in aluminium pans in nitrogen atmospheres, at 30–240 °C range with a heating rate of 10 °C/min.

X-ray diffraction analysis was conducted with Cu-K $\alpha$  radiation at a voltage and current of 40 kV and 30 mA, using X'pert Pro MPD (Philip, The Netherlands), to investigate the crystalline property of the EC microspheres formed at different temperature and CS film carrying EC microspheres. XRD patterns were recorded by monitoring diffractions from 5° to 50° and the scanning speed was 2°/min.

The mechanical property of CS film containing EC microspheres was evaluated through the measurement of the elongation and strain at break, according to the weight ratio of EC to CS. Tests were conducted with Shimadzu AUFOGRAPH testing system at 20 °C and a relative humidity of 45%. The initial gauge length was set to be 20 mm and the draw speed was set at 50 mm/min. In each measurement, samples of film were cut into 4 cm in length, and attached onto the two clamps of the machine. For reliability, five readings were taken for each type of the sample, and average value was used in this paper.

Equilibrium water uptake of five samples formed at different ratio of EC to CS was determined by measuring the extent of swelling of the matrices in water. To ensure complete equilibration, the samples were allowed to swell for 24 h. The excess surface-adhered liquid drops were removed by filter paper and the wet samples were weight 1 g using an electronic microbalance (Hangping, FA

2004, China). The waterish matrices were dehydrated in an oven at 60 °C until all the water was fully removed. The percent equilibrium water uptake was calculated as:

$$\left( \frac{\text{mass of swollen comatrices} - \text{mass of dry comatrices}}{\text{mass of dry comatrices}} \right) \times 100.$$

For actual data, five readings were taken for each type of the sample, and average value was used in the context.

### 3. Results

In this study, several testing methods were used to investigate the property of the CS film carrying EC microspheres (comatrix). The shape of EC microspheres in 2%, 4% and 6% CS solution altered remarkably. The figuration of EC microspheres in 6% CS solution prepared at different temperature varied modestly. The XRD patterns of them, however, transformed significantly. There was interaction occurred between CS and EC microspheres. The results of DSC, swelling studies and mechanical testing had altered obviously, according to different weight ratio of EC microspheres to CS.

#### 3.1. SEM observation

As can be seen in Fig. 2, with each increment of CS concentration, the microspheres' shape became rounder, smoother and smaller. The diameter of EC microspheres reduced from 2–15 (Fig. 2a) to 2–4  $\mu\text{m}$  (Fig. 2c). These results were consistent with Patrick B. O'Donnell and his co-workers' conclusion (O'Donnell & McGinity, 1997), namely, after the hydroxypropyl methyl cellulose's concentration in the aqueous phase being increased, the mean particle diameter of microspheres which were formed in solution of hydroxypropyl methyl cellulose decreased.

Fig. 3 shows that the change of shape and diameter at 40, 70 and 100 °C was not obvious, but the linkage between microspheres reduced with temperature. The diameter of microspheres prepared at 70 °C (3c) was slightly smaller than those fabricated at 40 and 100 °C (3f and g). Linkages between microspheres prepared at different temperature were observed even after being washed several times, but the microspheres prepared at 100 °C had less linkage than the others.

Results of SEM observation are shown in Fig. 4. EC microspheres dispersed uniformly in CS film, no occurrence of conglomeration. In Fig. 4c, the upper surface has some microspheres. In contrast, there are almost no microspheres on the lower surface. The thickness of the film is  $8.52 \pm 0.103 \mu\text{m}$ .

#### 3.2. FT-IR analysis

Some interaction, via the unsubstituted hydroxyl groups among molecules of EC and CS as well as hydroxyl groups

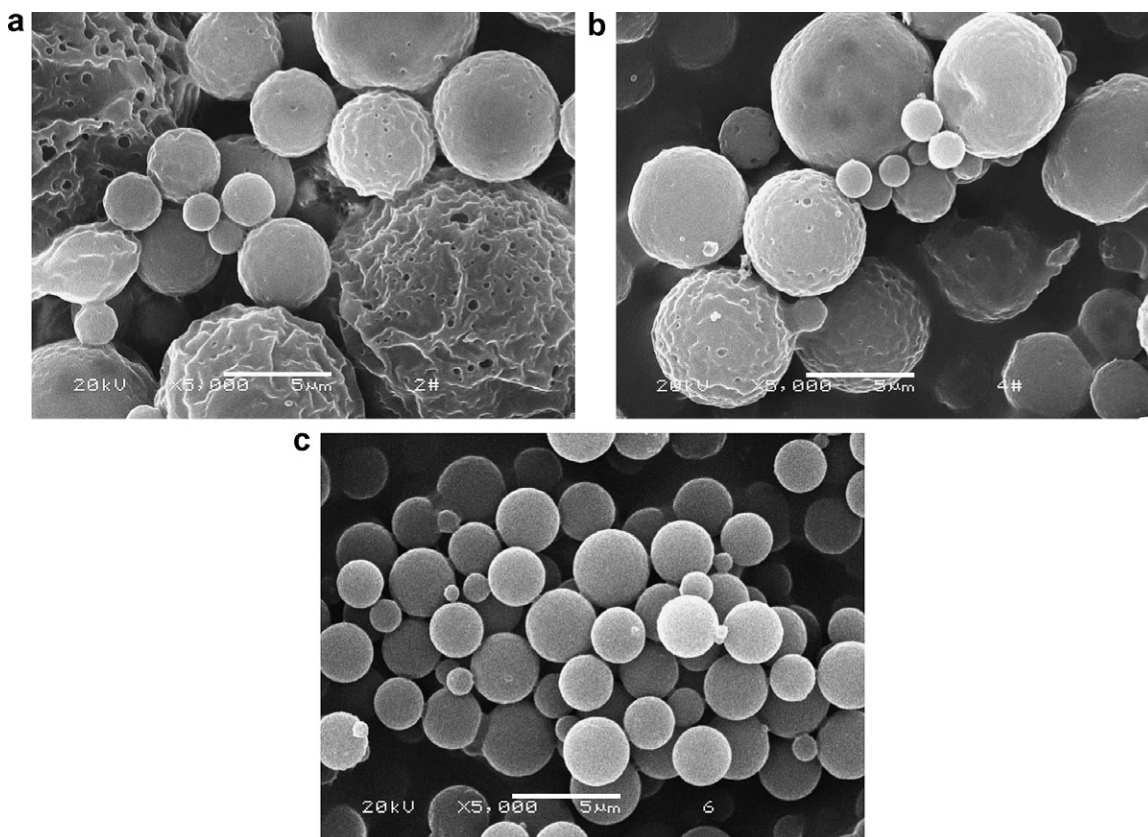


Fig. 2. SEM photographs of EC microspheres prepared in CS solution of different concentration (at 40 °C). (a) 2%; (b) 4%; (c) 6%.

of EC with ammonium groups of CS, occurred, which was confirmed by FT-IR spectra (Fig. 5). A band at  $3482\text{ cm}^{-1}$  was attributed to  $-\text{OH}$  stretching vibration in EC matrix, while a band at  $3435\text{ cm}^{-1}$  to  $-\text{NH}_2$  and  $-\text{OH}$  group stretching vibration in CS matrix. In the blending film, a shift from  $3453$  to  $3424\text{ cm}^{-1}$  was observed, the peak became wider, which indicated hydrogen bonding was enhanced. Fig. 5a also illustrated that a peak located at  $2870\text{--}3100\text{ cm}^{-1}$  different from the other samples (5b, c and d), may be attributed to hydrogen bonding formed between C–H and O–H group in CS and EC matrix.

### 3.3. Differential scanning calorimetry analysis

Fig. 6 shows the DSC curves of EC–CS physical mixture (6a) and CS film carrying EC microspheres (6b). Endothermic peaks were observed. The endothermic peak of EC–CS physical mixture was huge, attributing to water slopping over from the sample. In Fig. 6b, there are two endothermic peaks; the large one caused by water evaporating around  $100\text{ }^{\circ}\text{C}$ , the small one around  $146\text{ }^{\circ}\text{C}$  may be attributed to water escaping from EC microspheres.

Fig. 7 is DSC curves of EC microspheres prepared at different temperature. Besides the endothermic peaks of the three samples, each of the patterns had an exothermic peak at  $151.7\text{ }^{\circ}\text{C}$  (7a),  $147.6\text{ }^{\circ}\text{C}$  (7b) and  $151.8\text{ }^{\circ}\text{C}$  (7c).

The intensity of these crystallization exothermic peaks decreased with the preparing temperature increasing.

### 3.4. X-ray analysis

Fig. 8 shows XRD patterns of chitosan film carrying EC microspheres (8a), pure CS (8b) and pure EC (8c). All the peaks observed in these patterns were wide. In Fig. 8b, the peak found around  $15^{\circ}$  was assigned to chitosan chains aligned through intermolecular interactions, after CS film containing EC microspheres being formed, the peak vanished. Two major peaks around  $7^{\circ}$  and  $20^{\circ}$  of pure EC were observed in Fig. 8c. In Fig. 8a the peak of EC around  $7^{\circ}$  became wider and feebler, however, the peak around  $20^{\circ}$  was enhanced.

The intensity of crystallization peaks of EC microspheres prepared at different temperature was different. The crude EC was amorphous, but the pure EC microspheres formed at  $40\text{ }^{\circ}\text{C}$  had a sharp diffraction peaks around  $20^{\circ}$ ,  $28^{\circ}$  and  $35^{\circ}$  (Fig. 9a). Patrick B. O'Donnell and his coworkers had pointed out that when the solvent removed or evaporated from internal phase of PLA quickly, there were no crystalline PLA in contrast to eliminate the solvent slowly (O'Donnell & McGinity, 1997). In Fig. 9, with increment of temperature, the crystallization degree of amorphous EC decreased significantly, probably



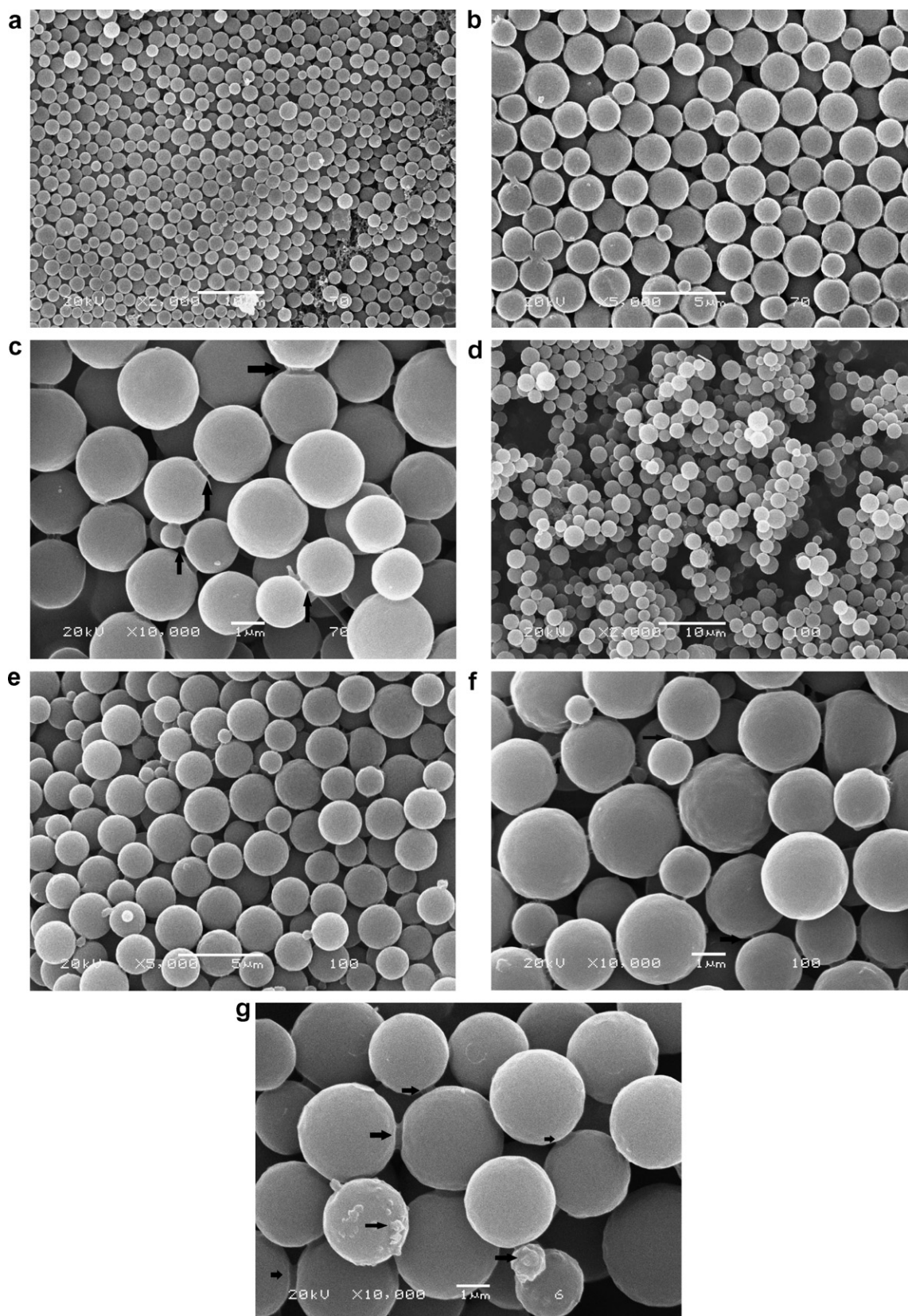


Fig. 3. Effect of different temperature on shape of EC microspheres in 6% CS solution: (a), (b) and (c), 70 °C; (d), (e) and (f) 100 °C; (g) 40 °C [(↑): linkage between microspheres].

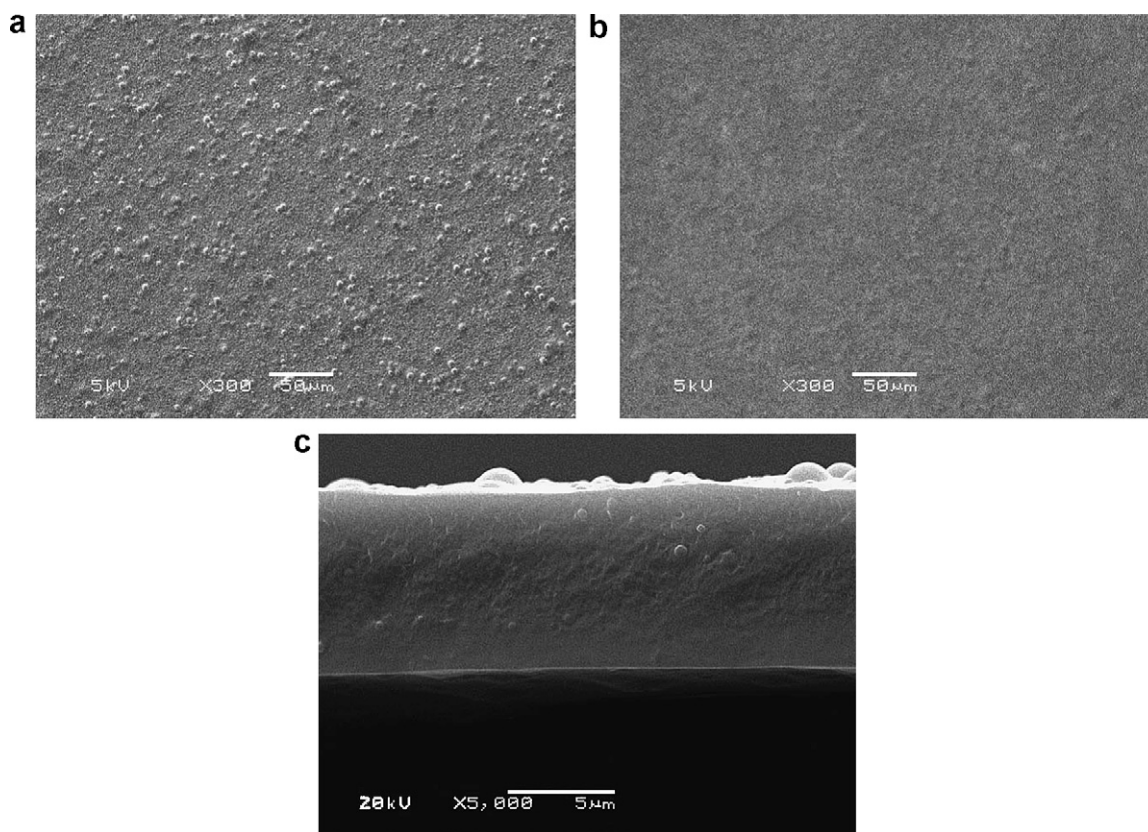


Fig. 4. SEM images of (a) upper and (b) lower surface of EC/CS film forming at 40 °C (6% CS solution). (c) The section of EC/CS film forming at 40 °C (6% CS solution).

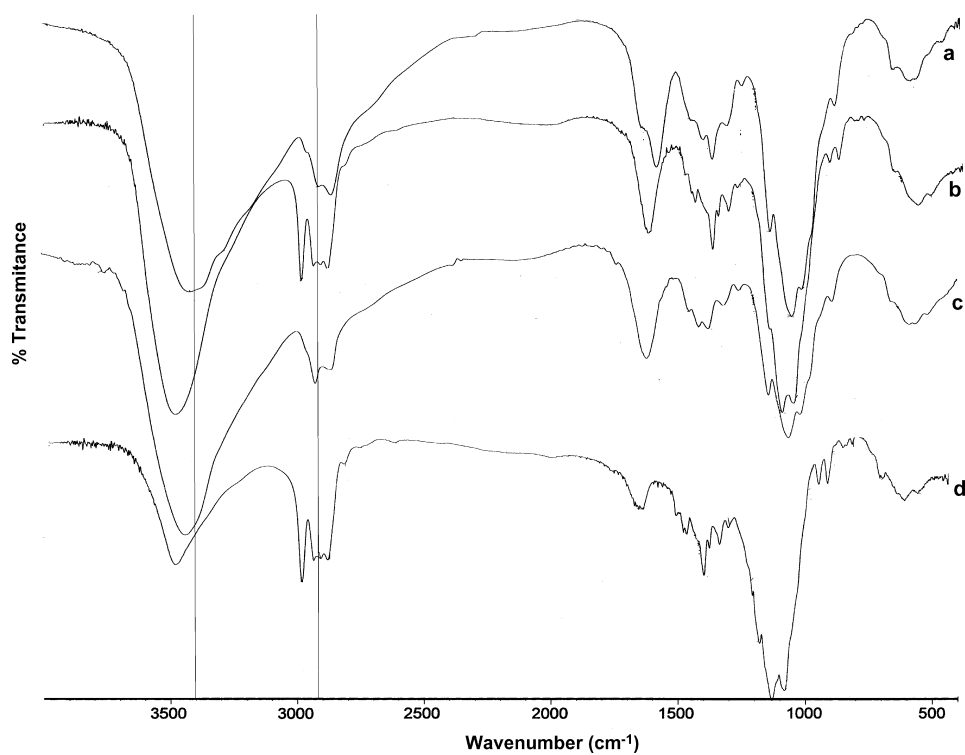


Fig. 5. FT-IR spectra of (a) EC/CS comatrix, (b) EC-CS physical mixture, (c) CS and (d) EC.

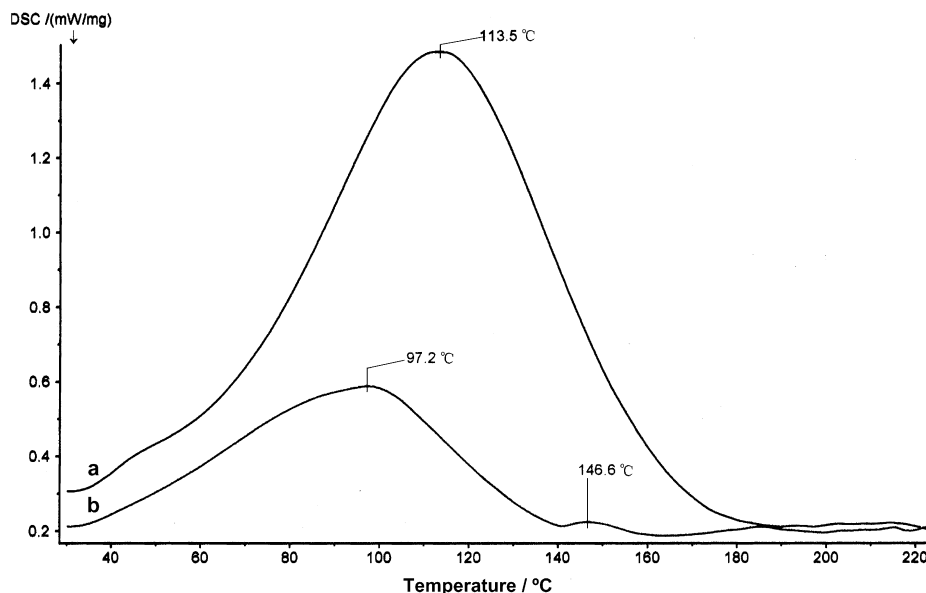


Fig. 6. DSC curves of (a) EC-CS physical mixture and (b) CS film carrying EC microspheres prepared at 40 °C.

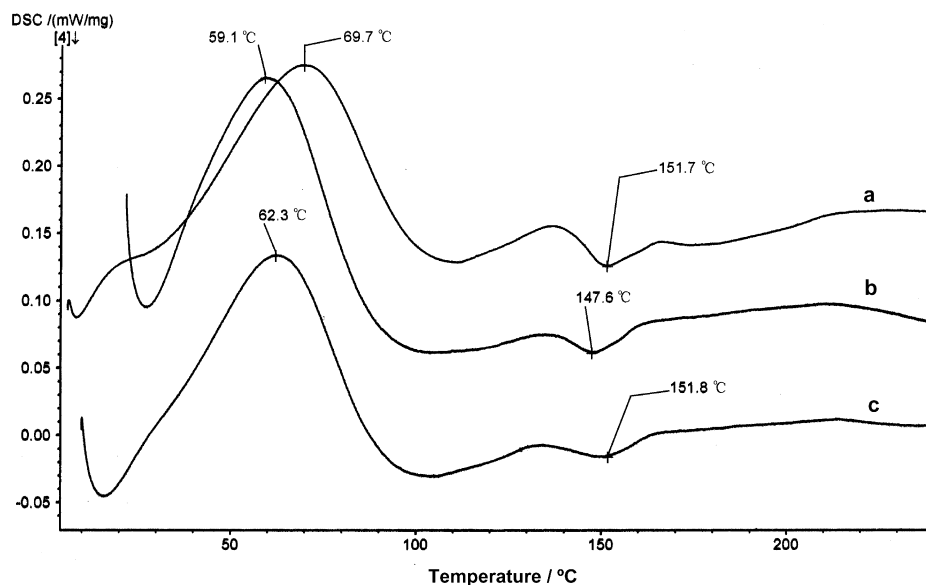


Fig. 7. DSC curves of EC microspheres prepared at different temperature: (a) 40 °C, (b) 70 °C, (c) 100 °C.

caused by organic solvent being removed so quick in high temperature that less time was left for EC crystallization.

### 3.5. Mechanical testing

EC microspheres in CS film could make a significant impact on CS film's property. In Fig. 10, all samples' strain at break decreased tremendously with each increment of EC microspheres in CS film. Moreover, only 0.5 g EC microspheres being combined with 6 g CS, the strain at break of the comatrix decreased obviously in both condi-

tion. In addition, dry samples' strain at break was much stronger than wet ones.

### 3.6. Swelling studies

The matrix water content is one of essential features for film carrying microspheres. The amount of water taken up by the hydrophilic component via the impregnation treatment was determined by the weight gain (Fig. 11). When the amount of water uptake of the comatrix is plotted versus the content of EC microspheres in CS film, its hydra-

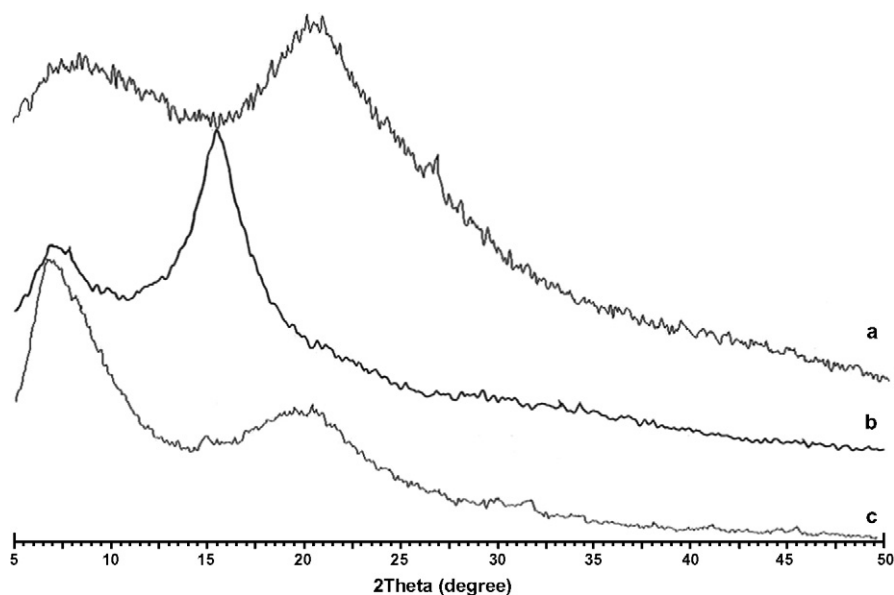


Fig. 8. XRD patterns of (a) CS film carrying EC microspheres (40 °C), (b) pure CS and (c) pure EC.

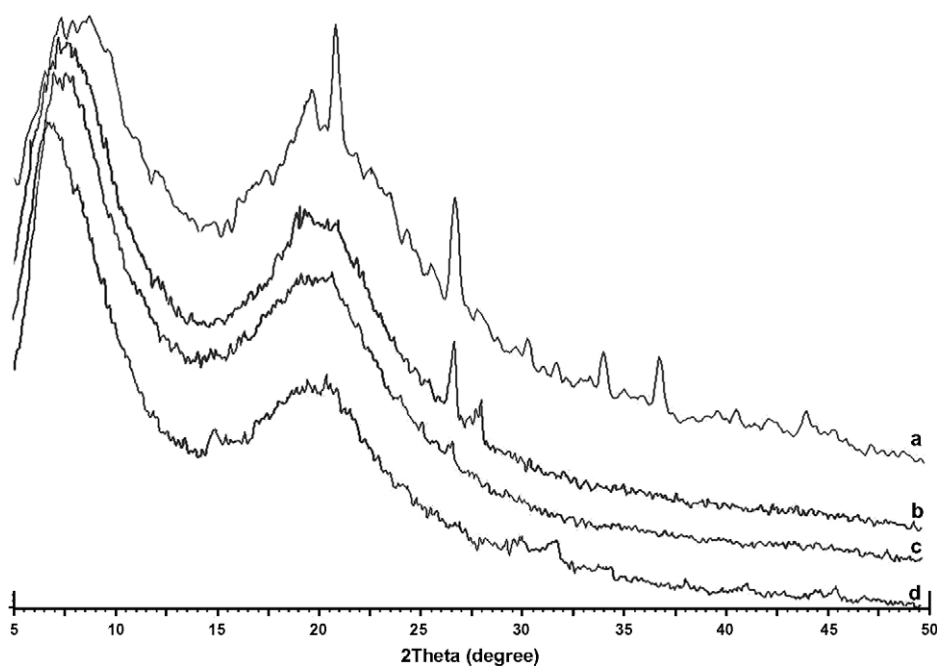


Fig. 9. XRD patterns of EC microspheres prepared in 6% CS solution at different temperature: (a) 40 °C, (b) 70 °C, (c) 100 °C and (d) pure EC power.

tion extent increased at first, and then its hydration extent decreased with the increment of EC microspheres. Despite pure CS film offered better water uptake ability than that of pure EC samples, the amount of water uptake of pure CS film was lower than that of CS film carrying EC microspheres.

#### 4. Discussion

In this study, CS film carrying EC microspheres is prepared and the influence of impact factors on the property of the comatrix is studied. The results show that the EC

microspheres disperse uniformly in CS film. It can obviously heighten the hydration extent in the comatrix, conversely, weaken its mechanical property. Its crystalline structure changes with different temperature.

The diameter and shape of microspheres prepared by solvent remove/solvent evaporation method are affected by several parameters, including the rate of stirring, the kind of solvent, solvent evaporation method, additives' concentration and the cubage of internal phase (Kawaguchi, 2000). In Fig. 2, EC microspheres prepared in 6% CS solution are round, small, uniform and smooth, caused by high viscosity of CS. When the concentration of CS



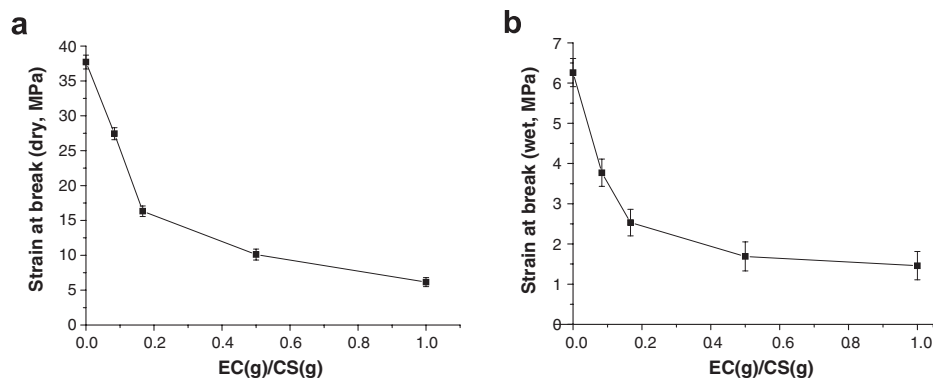


Fig. 10. Strain at break curve of CS film carrying EC microspheres as effect of weight ratio of EC microspheres/CS in dry (a) and wet (b) state.

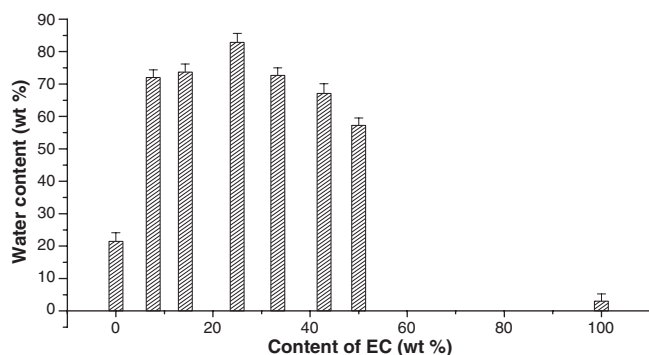


Fig. 11. The effect of EC microspheres content on water uptake of CS film carrying EC microspheres.

solution increases from 1% to 6%, its viscosity rises. The increasing of aqueous phase's viscosity corresponds to the viscosity decreasing of internal phase relatively, leading to the EC microspheres round in shape, smaller and smoother. The results are consistent with that of Patrick B. O'Donnell and McGinity (1997); who stated that along with the volume of internal phase expanding, as the polymer weight was kept constant, the microspheres' diameter had decreased. The smooth surface of EC microspheres can slow down the release of soluble drugs (Ruan, Feng, & Li, 2002).

The removal rate of solvents can influence the properties of microspheres significantly; solvent properties are critical in solvent extraction and solvent evaporation process, which are used to shape solid microspheres (Ruan et al., 2002). The solvent of EC in this study is ternary mixture consisting of  $\text{CH}_2\text{Cl}_2$ , methanol and acetone, the last two are soluble in water. In Fig. 2, the diameter of smooth EC microspheres forming in 6% CS solution is under  $3\ \mu\text{m}$ . By contrast, after their diameter expanding  $5\ \mu\text{m}$ , the surfaces of microspheres are coarse and porous. The larger microspheres have relatively small ratio of surface to volume, which block solvent moving out and cause highly porous skin. When EC solution is added into CS aqueous phase, the methanol and acetone with some  $\text{CH}_2\text{Cl}_2$  moves in the CS aqueous phase, causing fast precipitation of EC. The acetone in aqueous phase can reduce the interfacial ten-

sion between the organic phase and aqueous phase (Niwa, Takeuchi, Hino, Kunou, & Kawashima, 1993).

In Fig. 8, the XRD patterns illustrate that CS film carrying EC microspheres is more amorphous than pure CS and EC. The mechanical property of the comatrix decreases magnificently, probably caused by the intermolecular interactions of chitosan and ethyl cellulose chains aligning being disarranged, leading to their mechanical property decreasing, respectively. XRD patterns in Fig. 9 show that different crystalline structure in EC microspheres prepared at 40, 70 and  $100\ ^\circ\text{C}$ . When EC microspheres are prepared at  $40\ ^\circ\text{C}$ , the solvent evaporates relatively slow, its crystallization is enhanced. The slower solvent removal rate may give enough time to polymer molecules crystallization (Freiberg & Zhu, 2004).

Yang, Chung, Bai, and Chan (2000) Jeyanthi, Mehta, Thanoo, and DeLuca (1997) had point out that larger microspheres were formed because of higher internal viscosity at lower temperature as well as more materials were moved from the center of microspheres to outward at higher temperature. In Fig. 3c, f and g, the diameter difference of EC microspheres can be observed, the microspheres prepared at  $70\ ^\circ\text{C}$  are smaller than those formed at 40 and  $100\ ^\circ\text{C}$ . When EC microspheres prepared at  $40\ ^\circ\text{C}$ , methanol and acetone move out fast but their evaporation speed is slow, the internal phase becomes viscous gradually, leading to larger microspheres. Analogously, when EC microspheres prepared at  $100\ ^\circ\text{C}$ , the temperature is above boiling point of methanol, acetone and  $\text{CH}_2\text{Cl}_2$ , these solvents evaporating quickly, putting EC out from the center of microspheres, making larger microspheres with a hollow core. So,  $70\ ^\circ\text{C}$  is a proper temperature fitting for shaping EC microspheres in 6% CS solution.

Some linkages are found between microspheres (Fig. 3c, f and g) These linkages between the microspheres prepared at  $100\ ^\circ\text{C}$  are fewer than the others. This may be caused by less time being left for contacts of microspheres before they were solidified.

CS film carrying EC microspheres has much more water content than that of pure CS film, which was probably caused by hydrophilic improvement after the crystalline structure change of the comatrix. In Fig. 8a CS film carry-

ing EC microspheres is more amorphous than the others (8b and c), benefit for water penetrating. With the increment of EC over 25% in the comatrix, its swelling ability decreases, attributing to more EC being in the comatrix, more intermolecular interactions among EC molecules occurring, leading to hydrophilic ability decreasing.

In Fig. 6b, a small endothermic peak of CS film carrying EC microspheres at 146.6 °C has been observed, probably caused by water removing from EC microspheres in the process of crystallization exotherm of EC microspheres (melting microspheres). Furthermore, in Fig. 7a, an exothermic peak at 151.7 °C may connect to melting microspheres.

## 5. Conclusions

The EC microspheres disperse uniformly in CS film, having round shape and diameter between 0.5 and 3 µm. EC microspheres and CS film have interaction confirmed by FT-IR. The crystallization of EC alters at various temperatures. The EC/CS comatrix has remarkable water uptake ability and offers a new kind of composite film material for biomedical applications.

This article is restricted to investigate the preparation process and properties of CS film carrying EC microspheres including crystallization, shape and interaction. Further study should be focused on drug release property, tissue biocompatibility and cell proliferation of the comatrix.

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