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Fabrication of ethyl cellulose microspheres: Chitosan solution as a stabilizer

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Abstract−Ethyl cellulose was dissolved in ternary mixture, which consisted of 10 ml dichloroethane, 20 ml methanol and 10 ml acetone. Then, the ethyl cellulose microspheres were shaped in 1-6% chitosan solution and at 40 °C, 70 °C and 100 °C, respectively. The characteristics of these ethyl cellulose microspheres were also investigated based on the characterization of XRD, SEM, IR and DSC. The results showed that the most suitable conditions for ethyl cellulose microsphere preparation were in 6% chitosan solution and at 70 °C, in which the ethyl cellulose microsphere prepared were small and uniform. Moreover, from the XRD, IR and DSC data, the crystallization of ethyl cellulose microspheres decreased with the increase of the temperature; on the other hand, there some interactions happened between ethyl cellulose and chitosan, which shows a strong evidence for the intermolecular interactions and good molecular compatibility between ethyl cellulose and chitosan.

Key words: Ethyl Cellulose, Microsphere, Chitosan, Characterization

INTRODUCTION

Microspheres are well-known drug delivery vehicles. There are many polymers that have been shaped into microspheres, such as synthetic compounds (polyacrylates, -methacrylate and methacrylesters), semi-synthetic compounds (methyl-, ethyl-, hydroxycellulose) and natural compounds (protein, chitosan and alginate), among which ethyl cellulose (EC) is an outstanding material. EC can clearly slow down the rate of drug release rate, and is especially effective for water-soluble drugs. Using polyvinyl alcohol (PVA), alginate, pectin and gelatin as stabilizers, the EC microspheres can obviously decrease the diffluence speed of diclofenac sodium [1]. When EC matrix carries dimenhydrinate, the content of ethyl cellulose goes up, resulting in the decrease of drug release rate [2]. In addition, Pérez-Martınez and his co-works used EC microspheres to carry norfluazon, the releasing speed of which was obviously slower than that in the other carriers [3].

Ethyl cellulose's main chain is C-O-C. Its side groups also have a structure of the form C-O-C. The slight polar nature of the polymer is due to the difference in electronegativity of the main chain and the side groups. The glass transition temperature (T_g) reported for EC lies between 60 and 75 °C, and the softening and melting temperatures are in the range 135-150 °C and 165-175 °C, respectively [4].

As cellulose, chitin is a highly abundant polysaccharide in the world, with annual regrowing rates by far superior to the industrial production of synthetic polymers, and chitosan is the most important derivate of chitin [5].

Chitosan (CS) is a polysaccharide formed primarily of repeating units of b-(1-4)2-amino-2-deoxy-D-glucose (D-glucosamine). Moreover, the animated CS expresses excellent absorptive ability, which has been reported [6]. Generally, chitosan is soluble in aqueous medium in the presence of a small amount of acids such as acetic acid (AcOH), lactic acid, hydrochloric acid (HCl) and so on. Though the chitosan dissolves in aqueous acidic medium below pH 6.5, it precipitates above this pH by the addition of alkali solution like aqueous sodium hydroxide (NaOH). The application of chitosan was limited owing to the insolubility at neutral or high pH region.

Although the number of articles focusing on microspheres has increased quickly, little research has been done on EC microspheres which are shaped in chitosan solution. In this study, EC is dissolved in acetone-methanol-CH_2Cl_2 ternary mixture. The EC microspheres, then, are made by solvent remove/solvent evaporation method in CS solution.

EXPERIMENTAL

1. Materials

Ethyl cellulose was purchased from Niansha Chemical Reagent Company of Kunshan, Jiangshu, China. Chitosan was supplied by Haidebei Technological Co. Ltd of Shandong Province, China. Methanol, acetone and glacial acetic acid were supplied by Changlian Chemical Reagent Co. Ltd of Chengdu, China. Dichloroethane (CH_2Cl_2) was from Chemical Reagent Factory of Tianjin, China. Other reagents used here were analytical grade.

2. Microspheres Preparation

Ethyl cellulose microsphere preparation in chitosan solution of various concentrations. EC (0.5 g) was dissolved in ternary mixture consisting of 10 ml dichloroethane, 20 ml methanol and 10ml acetone. Different chitosan solution (1-6%, w/v) was prepared by dissolving 1, 2, 3, 4, 5 and 6 g chitosan in 100 ml diluted acetic acid (3% v/v), respectively. The primary oil/water emulsion (set as O/W) was obtained by injecting 40 ml EC solution in 100 ml chitosan aqueous solution. Emulsification was performed at room temperature under continuous stirring at 1,000 rpm for 30 minutes. The emulsion, then, was put in water incubator for 3 hours at 40 °C. After the organic solvent was evaporated, the microspheres were separated by centrifugation, fully washed with deionized water and lyophilized.

Heat-treated ethyl cellulose microspheres prepared in chitosan

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solution: 40 ml of the above-mentioned ternary solution containing 0.5 g EC was injected in 100 ml 6% CS solution with stirring at 1,000 rpm for 30 min. This step was repeated 3 times. After the three O/W emulsions were formed, they were heated in water bath at 40 °C, 70 °C and 100 °C, respectively, until CH_2Cl_2, methanol and acetone evaporated completely. When they were solidified, EC microspheres were separated by the methods mentioned above.

3. Characterization

The EC microspheres shaped under various conditions 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.

Infrared (IR) spectra of ethyl cellulose, chitosan, chitosan-ethyl cellulose (CS-EC) physical mixture and EC microspheres were recorded with an FT/IR spectrophotometer (American Perkin Elmer Co.). Samples were scanned from 500 cm^{-1} to 4,000 cm^{-1}.

X-Ray diffraction analysis was conducted with Cu-Kα 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.

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

RESULTS AND DISCUSSION

1. SEM Observation

In Fig. 1, with the increase of CS concentration, the microspheres gradually shape to the perfect sphere. The surface smoothness of the microsphere also improved. The size of EC microspheres was decreased. The diameter of EC microspheres was reduced from 2-15 μm (Fig. 2(a)) to 2-4 μm (Fig. 2(f)). The average particle diameter of microspheres formed in solution of hydroxypropyl methyl

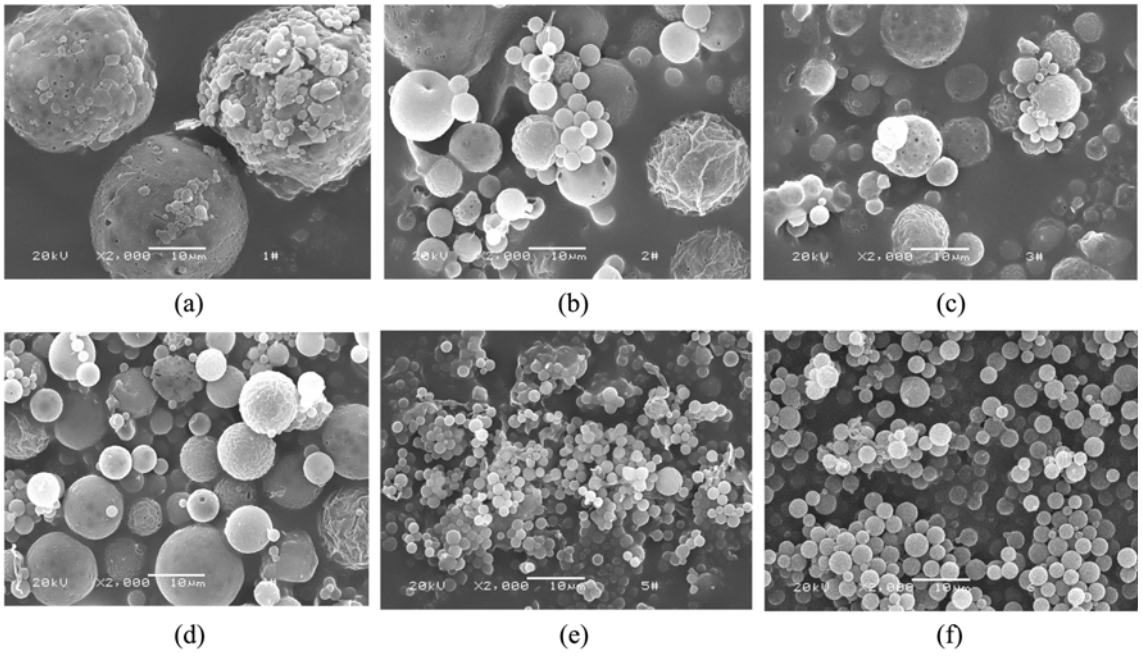

Fig. 1. SEM micrographs of EC microspheres prepared in CS solution of different concentrations (at 40 °C). (a) 1%; (b) 2%; (c) 3%; (d) 4%; (e) 5%; (f) 6%.

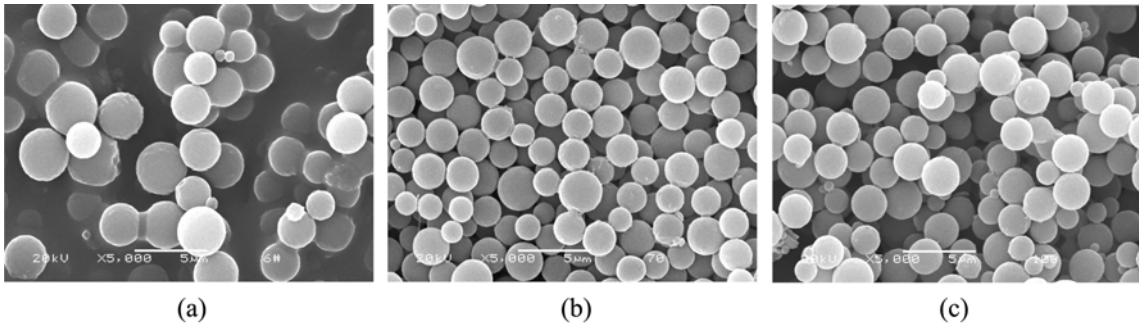

Fig. 2. Effect of different temperature on shape of EC microspheres in 6% CS solution: (a) 40 °C; (b) 70 °C; (c) 100 °C.

cellulose decreased with the increase of the hydroxypropyl methyl cellulose's concentration in the aqueous phase. These results were consistent with O'Donnell and his co-works' conclusion [7].

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 the internal phase [8]. In Fig. 1, EC microspheres prepared in 6% CS solution are round, small, uniform and smooth, caused by high viscosity of CS. When the concentration of CS solution increases from 1% to 6%, the solution's viscosity rises. The increase of the aqueous phase's viscosity corresponds to the viscosity decrease of internal phase relatively, leading to EC microspheres round in shape, smaller and smoother. The results are consistent as reported by O'Donnell et al. [7], stating that along with the volume of the 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 [9].

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 [9]. The solvent for EC in this study is a ternary mixture consisting of CH_2Cl_2, methanol and acetone. The last two are soluble in water. In Fig. 1, the diameter of the smooth EC microspheres formed in 6% CS solution is under 3 μm. By contrast, as their diameter expands to 5 μm, the surfaces of microspheres are coarse and porous. The larger microspheres have relatively small ratio of surface to volume, which blocks solvent moving out and causes highly porous skin. When EC solution is added into CS aqueous phase, the methanol and acetone with some CH_2Cl_2 move in the CS aqueous phase, causing fast precipitation of EC. The acetone in aqueous phase can reduce the interfacial tension between the organic phase and aqueous phase [10].

Fig. 2 shows that the change of shape and diameter at 40 ºC, 70 ºC and 100 ºC was not obvious, but the connectivity between microspheres reduced with increase of temperature. The diameter of microspheres prepared at 70 ºC (b) was slightly smaller than those fabricated at 40 ºC and 100 ºC (a and c). In addition, the diameters of these microspheres were 1-5 μm at 40 ºC, 0.5-3.5 μm at 70 ºC and 0.5-4 μm at 100 ºC, respectively.

Yang et al. and Jeyanthi et al. [11,12] pointed out that larger microspheres were formed because of higher internal viscosity at lower temperature, which let the organic solvent volatilize slowly, and the internal phase become more and more viscous before the microspheres were formed. Also, more materials were moved from the center of the microspheres to outward at higher temperature because of the higher volatilization rate of organic solvent pushing out the material from the core to the outside of the microspheres. In Fig. 2 the diameter difference of the EC microspheres can be observed; the microspheres prepared at 70 ºC are smaller than those formed at 40 ºC and 100 ºC. When EC microspheres were prepared at 40 ºC, methanol and acetone moved out fast but their evaporation speed was slow; the internal phase became viscous gradually, higher internal viscosity leading to larger microspheres. Similarly, when EC microspheres were prepared at 100 ºC, which is higher than the boiling point of methanol, acetone and CH_2Cl_2, the solvents evaporated quickly, pushing EC out from the center of microspheres, resulting in larger microspheres with a hollow core than the one prepared at 70 ºC. Thus, 70 ºC is a proper temperature for shaping EC microspheres in 6% CS solution.

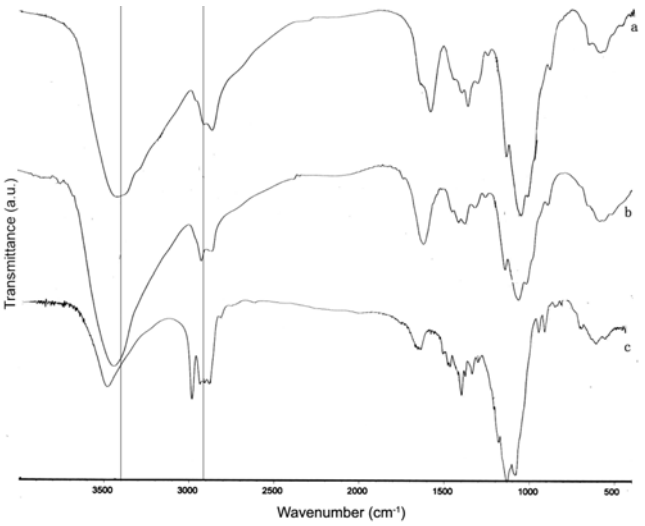

Fig. 3. FT-IR spectra of (a) EC microspheres, (b) CS and (c) EC.

2. FTIR Analysis

Some interaction, via the unsubstituted hydroxyl groups among molecules of EC and CS as well as hydroxyl groups of EC with ammonium groups of CS, occurred, which was confirmed by FTIR spectra (Fig. 3). A band at 3,482 cm^{-1} was attributed to -OH stretching vibration in EC matrix, while a band at 3,435 cm^{-1} to -NH_2 and -OH group stretching vibration in CS matrix. In the EC microspheres, a shift from 3,453 cm^{-1} to 3,424 cm^{-1} was observed, and the peak also became wider, indicative of enhanced hydrogen bonding.

3. XRD Analysis

The intensity of crystallization peaks of EC microspheres prepared at different temperatures was different. The crude EC was amorphous, but the pure EC microspheres formed at 40 ºC had sharp diffraction peaks around 20, 28 and 35 degree [Fig. 4(a)]. O'Donnell

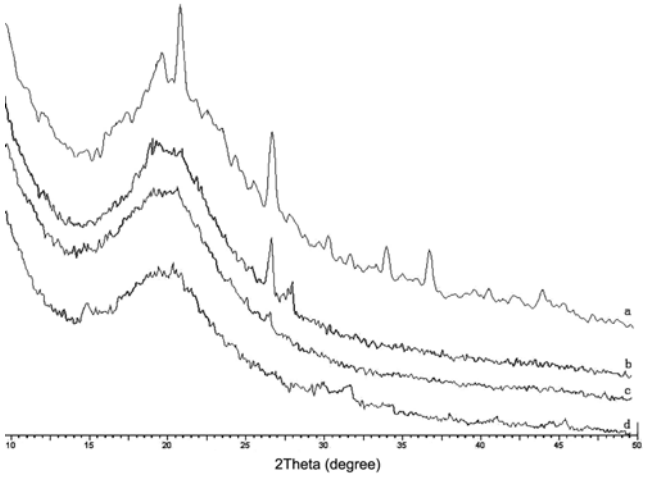

Fig. 4. XRD patterns of EC microspheres prepared in 6% CS solution at different temperatures: (a), 40 ºC; (b), 70 ºC; (c), 100 ºC and (d), pure EC power.

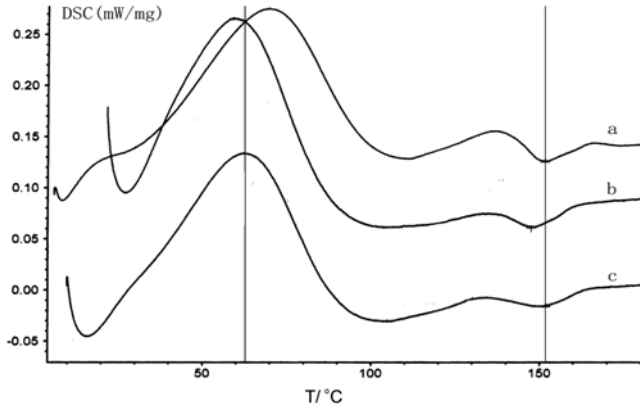

Fig. 5. DSC curves of EC microspheres prepared at different temperatures: (a) 40 °C; (b) 70 °C; (c) 100 °C.

and his coworkers pointed out that when the solvent was removed or evaporated from internal phase of polylactic acid (PLA) quickly, there were no crystalline PLA in contrast to eliminating the solvent slowly [12,13]. In Fig. 4, with increment of temperature, the crystallization degree of amorphous EC decreased significantly, probably caused by organic solvent being removed so quickly at high temperature that less time was left for EC crystallization. The slower solvent removal rate gives enough time for polymer molecules crystallization [14].

4. DSC Analysis

Fig. 5 shows the DSC curves of EC microspheres prepared at different temperatures. Besides the endothermic peaks of the three samples, each of the patterns have an exothermic peak at 151.7 °C (a), 147.6 °C (b) and 151.8 °C (c). The intensity of these crystallization exothermic peaks decreases with increasing preparing temperature. Furthermore, in Fig. 5(a), an exothermic peak at 151.7 °C maybe corresponding to melting microspheres is observed, which indicates that the crystallization of microspheres made at 40 °C is much stronger than at 70 °C and 100 °C. The results are also proved by XRD patterns.

CONCLUSION

The EC microspheres made in 6% CS solution and at 70 °C have round shape and diameter between 0.5 and 3 μm, which would be excellent carriers for various drugs. The interactions between CS and EC are confirmed by FT-IR, and the crystallization of EC is altered at various temperatures.

This study is restricted to investigating the preparation process and properties of EC microspheres including crystallization, shape and interaction. Further study should focus on drug release property, tissue biocompatibility and cell proliferation.

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