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Structural and morphological studies on poly(3-hydroxybutyrate acid) (PHB)/chitosan drug releasing microspheres prepared by both single and double emulsion processes

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Abstract

Drug releasing microspheres of poly(3-hydroxybutyric acid)/chitosan (PHB/CTS) with various compositions have been synthesized by both single and double emulsion methods, and collected by a freeze-drying process. In this study, gentamicin was used as an antibacterial medicine coated with PHB. The PHB/CTS microspheres of various compositions prepared by a single emulsion process (SEP) were identified as the major PHB phase together with a minor unknown Phase X by X-ray diffraction (XRD) and FT-IR. However, in the microspheres prepared using a double emulsion process (DEP) the dominant Phase was X and the minor phase was PHB. The size of the PHB/CTS microspheres prepared by SEP increased with the PHB/CTS ratio from 1 μm for 1:1 to 2 μm for 5:1. However, the size of the PHB/CTS microspheres prepared by DEP decreased with the PHB/CTS ratio from 1 μm for 1:1 to 800 nm for 5:1.

Keywords: Polymers, elastomers and plastics; Chemical synthesis; X-ray diffraction

1. Introduction

Delivering an effective antimicrobial at a sufficiently high concentration to the area of infection in combination with surgery is currently of considerable interest in controled release systems for the promotion of wound healing [1,2].

Bacterial poly(3-hydroxybutyrate acid) (PHB) is a well-known natural thermal-plastic polyester with biocompatibility and optical activity [3]. However, PHB has several inherent deficiencies in use, including brittleness and thermal instability [4]. Chitosan (poly(β-(1-4)-2-amino-2-deoxy-D-glucopyranose), CTS) is a cationic polysaccharide derived from the deacetylation of chitin [5]. Its biodegradation, biocompatibility and low toxicity lead to its being widely utilized for antibacterial spreading [6].

Since PHB does not have any functional groups usable for chemical modification, PHB-based materials are often blending with CTS [7]. PHB and CTS are both biodegradable for drug delivery. However, most drug delivery systems are made of films [7] or large scale spheres [2]. In this study, spheres in the submicron size range are synthesized to enhance the effect of drug delivery.

The PHB/CTS microspheres with various ratios were synthesized by both single and double emulsion methods and collected by a freeze-drying process. Gentamicin was used as an antibacterial medicine in this study. The effects of emulsion processes on the morphology and crystal structure of the drug delivering microspheres were investigated.

2. Experimental

The solutions used in the emulsion processes were as follows: (1) 1–5 g poly(3-hydroxybutyrate acid) (M_w = 380,000, Aldrich Chemical, USA) in 100 ml of methylene chloride, (2) 1 g chitosan (M_w = 400,000, Fluka, Steinheim, Germany) in 100 ml 1% acetic acid aqueous solution, (3) 1 g gentamicin (GTM, Sigma–Aldrich, Steinheim, Germany), (4) 1 g poly(vinyl alcohol) (PVA, Sigma Chemical Co., St. Louis, MO) in 100 ml of DI water and (5) 0.5 g PVA in 500 ml of DI water.

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The single emulsion process (SEP) was carried out by mixing the PHB and the CTS solutions firstly in an agitator for 20 min with the PHB:CTS ratios of 1:1, 3:1 and 5:1, and then mixed with the gentamicin solution by ultrasonic agitation for 5 min. The mixture was then put in the PVA solution (4) by syringe at a rotation rate of 600 rpm for 24 h, and the microspheres were finally collected by a freeze-drying method.

For the double emulsion process (DEP), the SEP mixture is put again into the PVA solution (5) at a rotation rate of 600 rpm for 24 h and the microspheres then collected by a freeze-drying method.

The crystalline phases of the dried sample were examined using X-ray diffraction (XRD) (Rigaku D-Max/IIIV, Tokyo, Japan) with a scanning speed of 4° min^{-1}. The operation tube voltage and current were 30 kV and 20 mA. The surface morphology and size distribution of the microspheres were examined by scanning electron microscopy (Hitachi S-3000N, Japan). The chemical behaviour and molecular bonding structure were evaluated by Fourier transform infrared spectroscopy (FT-IR Perkin-Elmer Spectrum One FT-IR spectrometer, Boston, USA) with a spectral resolution of 4 cm^{-1}, normalized to the spectrum of the blank KBr pellet.

3. Results and discussion

Fig. 1 is the XRD pattern of the microspheres synthesized by SEP with different PHB/CTS ratios. Fig. 1(a) is for pure CTS, showing only a reflection at 20.1°. In Fig. 1(b), the major phase PHB is identified for the ratio of 1:1 at 600 rpm. However, all the PHB, CTS and PVA have a reflection around 20° and no minor phase can be identified. With increasing PHB/CTS ratio from 1:1 to 5:1, the PHB reflections are clearer and shift toward the lower angle due to the improvement of the crystallinity. Compared with the pure PHB reflection in Fig. 1(e), a set of reflections between 29.0° and 57.0° that neither belong to PHB nor to CTS are thought to be the cross linked product of the PHB/CTS composite (hereafter called Phase X) in Fig. 1(b–d). The reflection intensity of the Phase X increases with the PHB/CTS ratio, revealing that the higher composite ratio of PHB/CTS stabilizes the X product.

Fig. 2 shows the XRD pattern of the microspheres synthesized by DEP with different PHB/CTS ratios and rotation speeds. In Fig. 2(a), the unknown Phase X is dominating with the PHB phase the minor for the ratio of 1:1 and 600 rpm. With increasing PHB/CTS ratio in Fig. 2(b and c), the PHB reflections steadily decrease and the X Phase increases. For the ratio of 5:1, the reflection at 19.5° corresponds to the PVA phase, revealing extra PVA content for DEP.

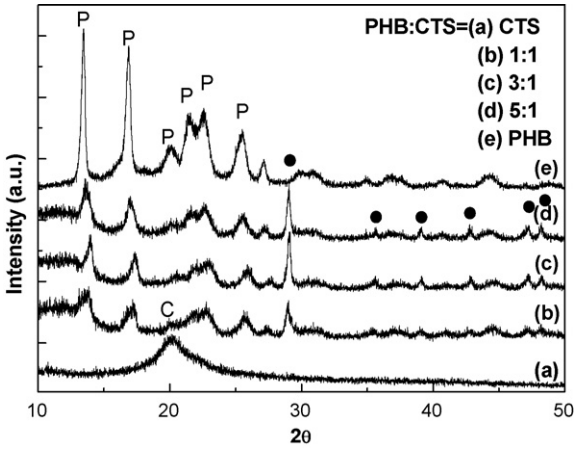

Fig. 1. XRD patterns of the PHB/CTS drug delivering microspheres with various prepared by SEP: (a) CTS pure and for PHB/CTS ratios, (b) 1:1, (c) 3:1, (d) 5:1 and (e) pure PHB (P: PHB, C: CTS, ●: Phase X).

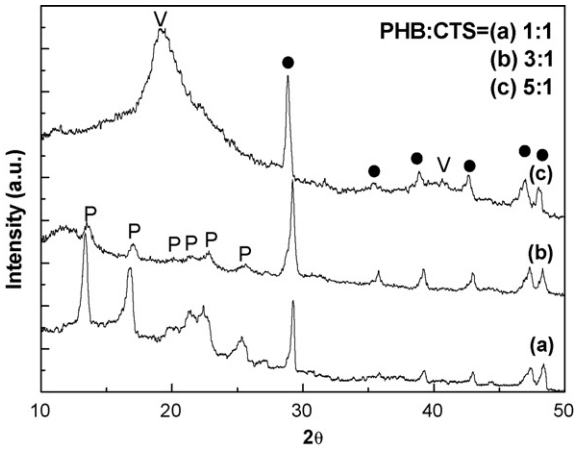

Fig. 2. XRD patterns of the drug delivering microspheres with various compositions prepared by DEP with various PHB/CTS ratios: (a) 1:1, (b) 3:1, (c) 5:1 (P: PHB, V: PVA, ●: Phase X).

The FT-IR spectra of the microspheres prepared by SEP are shown in Fig. 3. Fig. 3(a–c) show the spectra of the samples with the PHB/CTS ratios from 1:1 to 5:1, revealing absorption peaks at 1652 cm^{-1} for the $NHCOCH_3$ bonding of CTS and at about 1550 cm^{-1} for the $-NH_2$ group. The pure PHB spectrum is shown in Fig. 3(d), where the bands at 980, 1230, 1282 and 1728 cm^{-1} are shown to arise from the crystalline PHB phase and those at 1184 and 1741 cm^{-1} from the amorphous phase. The bands at 1741 and 1728 cm^{-1} are assigned to the stretching vibration of the amorphous and crystalline carbonyl groups, respectively. The band at about 1381 cm^{-1} is assigned to symmetric wagging of the CH_3 group, and the band at 1230 cm^{-1} is proposed as the conformational band of the helical chains, since no amorphous bands of the same group could be found. The bands at 1184 and 1133 cm^{-1} are characteristic of the asymmetric and symmetric stretching vibrations of the C–O–C group, respectively. The decrease of crystalline-sensitive bands of CTS

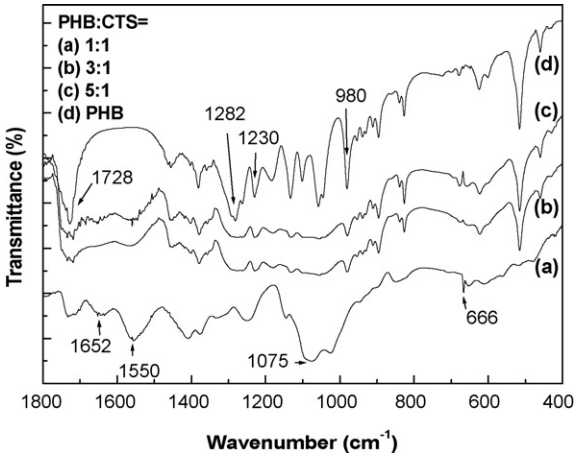

Fig. 3. FT-IR spectra of the PHB/CTS drug delivering microspheres prepared by SEP with various PHB/CTS ratios: (a) 1:1, (b) 3:1, (c) 5:1 and (d) pure PHB.

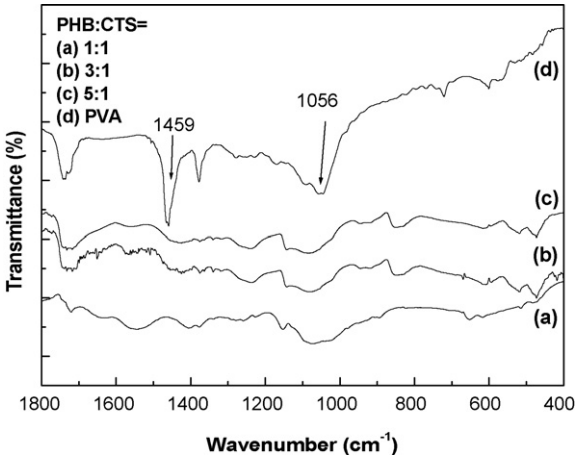

Fig. 4. FT-IR spectra of the PHB/CTS drug delivering microspheres prepared by DEP with various PHB/CTS ratios: (a) 1:1, (b) 3:1, (c) 5:1 and (d) pure PVA.

at 1075 and 666 cm^{-1} demonstrates that blending disturbed the crystallization of CTS [8]. With increasing composition ratio, the bands of the CTS give way to the PHB bands, with the stretching modes revealing bonding between PHB and CTS, which may correspond to the Phase X.

Fig. 4 shows the FT-IR spectra of the microspheres prepared by DEP. Fig. 4(a) shows the same spectrum as that by SEP with the PHB/CTS ratio of 1:1. With increasing composition ratio, the bands of PHB/CTS stretching modes give way to the PVA bands. The FTIR spectrum of the pure PVA membrane (Fig. 4(d)) shows the absorption peaks at about 1056 and 1459 cm^{-1} for the –C–O group. Compared with Fig. 3, with increasing content of PVA for DEP, the absorption peaks at 1640 cm^{-1} (NHCOCH3) and 1550 cm^{-1} for the $–NH_2$ group increase but the absorption peak at 1025 cm^{-1} for the –C–O–C group decreases [9].

The surface morphologies of the microspheres with different compositions by SEP and DEP are shown in Fig. 5. In Fig. 5(a), the microspheres of PHB/CTS = 1:1 prepared by SEP have average diameter of about 1 μm with rough edges. Melting and linkage between spheres are induced by heat from the electron beam. The microspheres prepared with the higher ratio of 5:1 are shown in Fig. 5(b) with larger average size of about 2 μm.

The morphologies of the microspheres prepared by DEP are shown in Fig. 5(c and d), revealing smaller size of about 1 μm and 800 nm at the ratios of 1:1 and 5:1, respectively, compared with those prepared by SEP. Higher PHB/CTS ratio results in more Phase X (Fig. 1) and induces larger microsphere size of SEP, but increasing Phase X with PVA content (Fig. 2) induces smaller particle size for DEP. The microspheres prepared by DEP show more rounded shape than SEP for the ratios of 1:1 and 1:5.

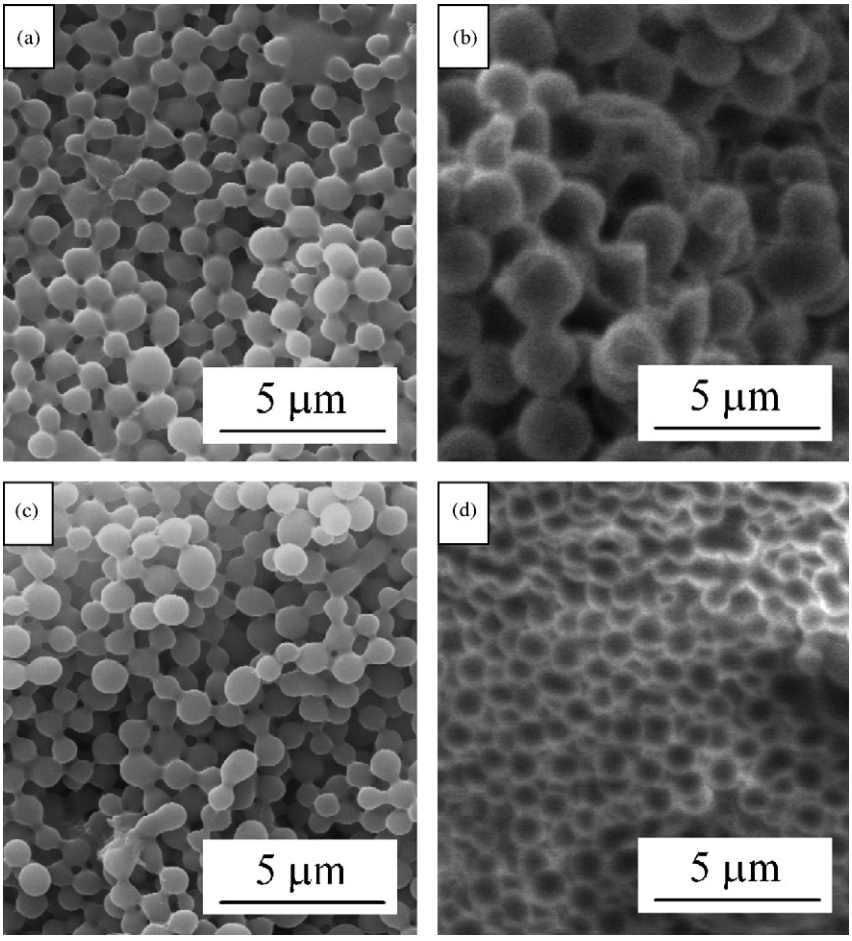

Fig. 5. SEM images of the PHB/CTS microspheres with various PHB/CTS ratios: (a) 1:1, (b) 5:1 by SEP, (c) 1:1 and (d) 5:1 by DEP.

4. Conclusions

The effects of emulsion processes on the PHB/CTS microspheres with various compositions synthesized by both single and double emulsion methods (SEP and DEP) and collected by a freeze-drying process have been investigated. With the increasing PHB/CTS ratio from 1:1 to 5:1 for SEP, the PHB reflections are clearer. Phase X increases, and the size of microspheres increases from 1 to 2 μm. However, when the PHB/CTS ratio increases from 1:1 to 5:1 for DEP, Phase X became the major phase with minor PHB and PVA appearing as identified by XRD and FT-IR. The size of microspheres prepared by DEP decreases from 1 μm for PHB/CTS = 1:1 to 800 nm for 5:1.

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