Preparation and Evaluation of Poly(3-hydroxybutyrate) Microspheres Containing Bovine Serum Albumin for Controlled Release

Yong-Liang Zhao, Feng Tian, Chang-Jun Liu, Fan Li, Nan Xing

Institute of Medical Equipment, Academy of Military Medical Science, Tianjin 300161, China

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ABSTRACT: The utility of the Poly(3-hydroxybutyrate) (PHB) to encapsulate and control the release of bovine serum albumin (BSA), via microspheres, was investigated. Various preparing parameters, including polymer concentration in oil phase, emulsification concentration in external water phase, volume ratio of inner water phase to oil phase, and volume ratio of primary emulsion to external water phase were altered during the microspheres production. The effects of these changes on the morphological characteristics of the microspheres, size of the microspheres, drug loading, encapsulation efficiency, and drug release rates were examined. The diameter of the microspheres ranged from 6.9 to 20.3 μm and showed different degrees of porous structure depending on the different preparation parameters. The maximum and minimum BSA encapsulation efficiency within the polymeric microspheres were 69.8 and 7.5%, respectively, varying with preparation conditions. The controlled release characteristics of the microspheres for BSA were investigated in pH 7.4 media. The initial BSA burst release from 8.9 to 63.1% followed by constant slow release for 28 days was observed for BSA from BSA-loaded microspheres and followed the Higuchi matrix model. So, the release behavior of microspheres showed the feasibility of BSA-loaded microspheres as controlled release devices. Pristine BSA, pristine PHB microspheres, and BSA-loaded microspheres were analyzed by Fourier transform infrared spectrophotometer, which indicated no interaction between BSA and PHB. Differential scanning calorimetry on BSA-loaded microspheres indicated a molecular level dispersion of BSA in the microspheres. J Appl Polym Sci 110: 3826–3835, 2008

Key words: PHB microspheres; double emulsion; controlled release; BSA

INTRODUCTION

Recently, many pharmacologically active peptides and proteins are becoming gradually good candidates for therapeutic drug treatment with the development of biotechnology and genetic engineering. However, their clinical applications in oral administration have been limited due to their higher molecular weight, which makes diffusion through biological membrane difficult, and their instability in the gastrointestinal environment. Alternative administration options by frequent injection are also tedious and expensive.[1] For these problems, priority is being given to developing controlled delivery systems with a greater parenteral dosing interval, which will increase patience acceptance, and improve drug management.

Injectable microspheres containing proteins or peptides as controlled release devices have been widely used for the treatment of human diseases and animal health. Fundamental understanding of the relationship among the size of microspheres, encapsulation efficiency, and protein release property is essential to design microspheres delivery systems. Double-emulsion solvent extraction/evaporation method is one of the most popular methods to encapsulate hydrophilic drugs, specially protein and peptide drugs, into microspheres.[2–4] Both natural and synthetic biodegradable polymers have been investigated for the controlled drug release.[5–8] Among these polymers, poly-3-hydroxybutyrate (PHB) is found to be remarkable for its application in drug delivery due to its excellent biocompatibility and biodegradability. Hydrolytic degradation of PHB *in vitro* proceed to the monomer, D-(−)-3-hydroxybutyric acid. This acid is a normal constituent of blood and, in common with acetoacetate and acetone, is one of the three ketone bodies which are produced endogenously by the process known as ketogenesis. It is therefore thought that PHB will be well tolerated *in vivo*. So, PHB is considered to be a good candidate for drug delivery.

Some researcher had studied the effects of PHB weight on the characteristics of microspheres.[9] Decrease in average molecular weight of PHB tended to reduce drug release from compacts containing tetracycline hydrochloride and polyhydroxybutyric acid (PHB) as controlled release devices.[10]

Correspondence to: F. Tian (tianfeng62037@yahoo.com.cn).

Progressively faster rates of drug release from P(HB-HV) copolymer matrices prepared by solvent casting and melt-processing were obtained on increasing the HV content.[11] The crystallization kinetics and morphology of P(HB-HV) polyesters both with and without the incorporation of a model drug, methyl red, had been investigated to influence drug release characteristics.[12] The release rate of the drug could be controlled over a wide range by proper choice of the kind and amount of additive in the preparation of the PHB microspheres.[13] The effect of various PHB/chitosan ratios on the morphology and crystal structure of the drug release microspheres was investigated.[14] However, there is few literatures focused on the effects of polymer concentration in oil phase, emulsification concentration in external water phase, volume ratio of inner water phase to oil phase, and volume ratio of primary emulsion to external water phase on the characteristics of PHB microspheres containing protein. This is the aim of the present article.

In this study, the microspheres are prepared with Bovine serum albumin (BSA) as a model drug by the double emulsion method and are characterized by various techniques. The microspheres sizes are measured by optical microscopy. Scanning electron microscope (SEM) is used to observe the surface morphology of microspheres. A Fourier transform infrared spectrophotometer (FTIR) is used to explore the interactions between BSA and PHB. The physical state of BSA inside the microspheres is assessed by using Differential scanning calorimetry (DSC). Among the properties of microspheres, the BSA release behavior of microspheres is important to confirm the feasibility of PHB microspheres containing BSA as controlled release devices.

MATERIALS AND METHODS

Materials

PHB (M_w = 437 kDa) was purchased from Tianlu Food (Tianjin, China). Poly(vinyl alcohol) (PVA) (98% hydrolyzed, M_w = 25 kDa) was purchased from Sinopharm Chemical Reagent (Shanghai, China). BSA (fraction V, 66 kDa) was purchased from Sigma Chemical Company. All other reagents were of reagent grade.

Microsphere preparation

Double-emulsion solvent evaporation method was used to prepare the microspheres. Briefly, certain volume of an aqueous solution of 30 mg/mL BSA (W1 phase) was added into PHB dissolved in chloroform (O phase). The mixture was emulsified using a homogenizer (FJ 200, Shanghai, China) at 23,000 rpm for 1 min. The resultant W1/O emulsion was added to certain volume of an aqueous solution of PVA and 0.9% NaCl (W2 phase) and homogenized at 13,000 rpm for 4 min. The resultant W1/O/W2 emulsion was then stirred for up to 4 h at 500 ± 10 rpm on a magnetic stirrer to allow the solvent to evaporate. The particles were collected by centrifugation in 5000 rpm for 20 min, washed three times in solution of 0.9% NaCl, and dried under vacuum at −40°C for 24 h. The particles were stored in a desiccator at 4°C before use.

Analyses of microspheres size

Freeze-dried microspheres were redispersed in distilled water and observed by microscopy (MicroStar, America).

Observation of surfacial morphology

The surface morphology of the polymeric microspheres was examined by SEM (Jsm-5600LV, Jeol, Japan) after the samples were coated with platinum.

Evaluation of the BSA loading and encapsulation efficiency

About 10-mg BSA-loaded microspheres were dissolved in chloroform with vigorous shaking at room temperature for 24 h. After dissolved completely, 10 mL physiological phosphate buffer (PBS) solution (pH 7.4, 0.1 mol/L) was added to dissolve the BSA in the microspheres. Then, the resultant solution was filtrated. After that 5-mL Coomassie brilliant blue was added into 0.5-mL resultant solution to be detected at 595 nm by UV/Vis spectrophotometer.

BSA loading and encapsulation efficiency were determined by eqs. (1) and (2), respectively:

$$\text{BSA loading (w/w \%)} = (\text{amount of BSA in microspheres})/\text{amount of microspheres}) \times 100 \quad (1)$$

$$\text{Encapsulation efficiency (w/w \%)} = (\text{retained BSA amount})/(\text{initially loaded BSA amount}) \times 100 \quad (2)$$

Interaction between BSA and polymer

A Fourier transform infrared spectrophotometer (FTIR, Perkin–Elmer Spectrum 2000) was used to explore the interactions between BSA and PHB using potassium bromide pellets.

The physical state of BSA inside the microsphere

The physical state of BSA inside the microspheres was assessed by differential scanning calorimetry

TABLE I
Preparation Conditions for BSA Microspheres

Formulation ID	PHB concentration (mg/mL)	PVA concentration (mg/mL)	W1/O	(W1/O)/W2
A	10	20	1 : 5	1 : 10
B	20	20	1 : 5	1 : 10
C	30	20	1 : 5	1 : 10
D	50	20	1 : 5	1 : 10
E	30	10	1 : 5	1 : 10
F	30	50	1 : 5	1 : 10
G	30	20	1 : 20	1 : 10
H	30	20	8 : 20	1 : 10
I	30	20	1 : 5	1 : 1
J	30	20	1 : 5	1 : 8
K	30	20	1 : 5	1 : 12

TABLE II
Effect of the Preparation Conditions on Sizes of the Microspheres

Formulation ID	Particle diamerer (μm)	SD (μm)
A	7.8	2.8
B	10.2	3.8
C	11.9	2.3
D	15	4.1
E	12.5	4.5
F	11.5	2.7
G	16.3	2.9
H	6.9	3.1
I	20.3	7.2
J	14.9	2.9
K	9.2	3.5

(DSC 822e, Mettler Toledo, Switzerland). The samples were sealed in aluminum pans with lids. The samples were purged with pure dry nitrogen at a flow rate of 2 mL/min. A temperature ramp speed was set at 10°C/min, and the heat flow was recorded from 40 to 225°C. Indium was used as the standard reference material to calibrate the temperature and energy scales of the DSC instrument.

In vitro BSA release

Microspheres (50 mg) were placed in triplicate into Eppendorf tubes and incubated in 10 mL release medium (PBS buffer, pH = 7.4 0.1 mol/L) under agitation (100 rpm) at 37 ± 0.5°C. At desirable time intervals, the microspheres suspension was centrifuged at 5000 rpm for 20 min. The supernatant (10 mL) was withdrawn and replaced with 10-mL fresh release medium. The amount of BSA released was determined by measuring the BSA content in the supernatant. To determine the BSA content in the supernatant, 5-mL Coomassie brilliant blue was added into 0.5-mL supernatant solution to be detected at 595 nm by UV/Vis spectrophotometer.

RESULTS AND DISCUSSION

This study was carried out to explore the feasibility of preparing BSA microspheres using the double-emulsion evaporation method. Generally speaking, the characteristics of the microspheres such as their particle sizes, morphology, loading, encapsulation efficiency, and vitro release behavior are affected by the different experimental conditions. In the present study, several parameters such as PHB concentration in the oil phase, PVA concentration in the external water phase, volume ratio of inner water phase to oil phase (W1/O), and volume ratio of primary emulsion to external water phase [(W1/O)/W2] are explored to confirm their effects on the characteristics of BSA-loaded microspheres. The preparation parameters used in this study are listed in Table I.

Effect of preparation conditions on size, morphology, loading and encapsulation efficiency of the microspheres

Effect of the PHB concentration in the oil phase

Polymer concentration is a key factor to influence the characteristics of microspheres. As shown in Table II (formulation A, B, C, and D) and Table III (formulation A, B, C and D), Generally, the viscosity of a polymer solution has a significant effect on the sizes of the resultant microspheres. The more viscous is the polymer solution, the more difficult it is to break it down into smaller droplets, leading to bigger microparticles.[15] Clearly, the PHB concentration had a greater impact on particle sizes. When the PHB concentration increased from 10 to 50 mg/mL, the average sizes of PHB microspheres increased from 7.8 to 15 μm (Table II and Fig. 1) due to the

TABLE III
Characteristics of BSA-Loaded Microspheres[a]

Formulation ID	A	B	C	D	E	F	G	H	I	J	K
BSA loading (%)	5.6	4.5	3.0	4.1	1.3	2.1	3.8	4.1	4.1	2.9	3.2
Encapsulation efficiecy (%)	7.5	22.1	36.4	56	13.6	29.5	61.2	17.2	69.8	52.9	20.4
Initial BSA burst release (%)	63.1	49.5	39.3	8.9	57.0	49.6	12.4	57.1	20.1	22.2	47.0

[a] For sample characteristics in Table I. Initial BSA burst (%): BSA released during the first 24 h.

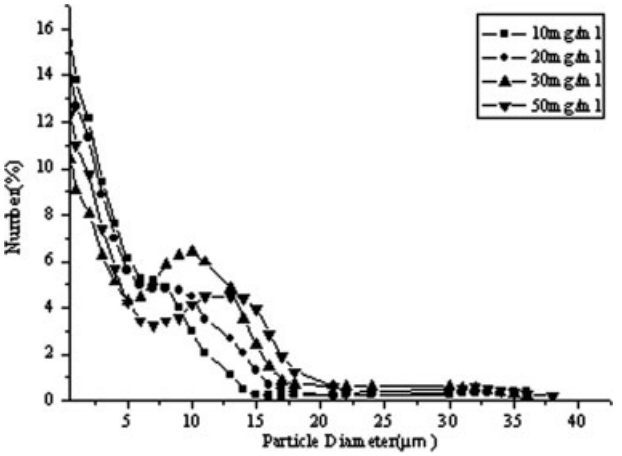

Figure 1 Effect of PHB concentration on particle size of microspheres.

increase in viscosity of the polymer solution. Meanwhile, the solidification of microspheres was more rapid at a higher polymer concentration, which might result in a viscous polymer layer at the microsphere droplet. It hindered the diffusion of BSA to the external water phase. As a consequence, a higher polymer concentration yielded a higher encapsulation efficiency of BSA.

Figure 2(A–D) shows the surface structure of the microspheres prepared with different PHB concentrations. It can be seen that their surface morphology differs obviously. The surface of microspheres prepared with higher PHB concentrations is less porous than that of the lower PHB concentrations. This phenomenon could be explained by as follows. The microspheres prepared with higher PHB concentration hardened more quickly during the preparation procedure. The reason for the porous surface of the microspheres was that solvent had evaporated too quickly from the microspheres.

Effect of PVA concentration in the external water phase

It is well known that the particle size can be controlled by means of varying the emulsifier concentrations in the external water phase.[3,16] In the present work, 10, 20, and 50 mg/mL PVA solutions are used

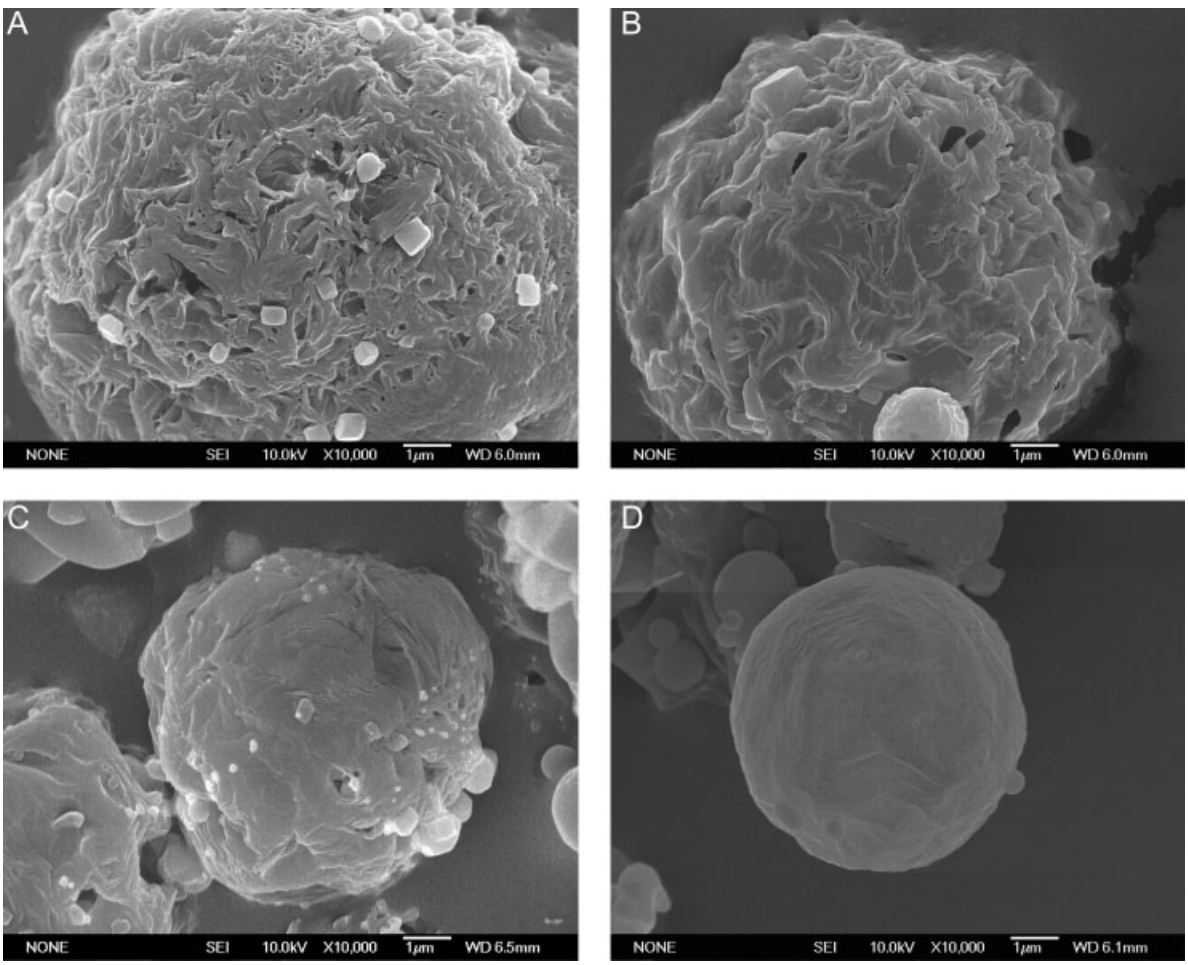

Figure 2 Surface of PHB microspheres prepared with different PHB concentrations. Caption: A, B, C, and D represent 10, 20, 30, and 50 mg/mL PHB, respectively. Size of the bar is 1 μm.

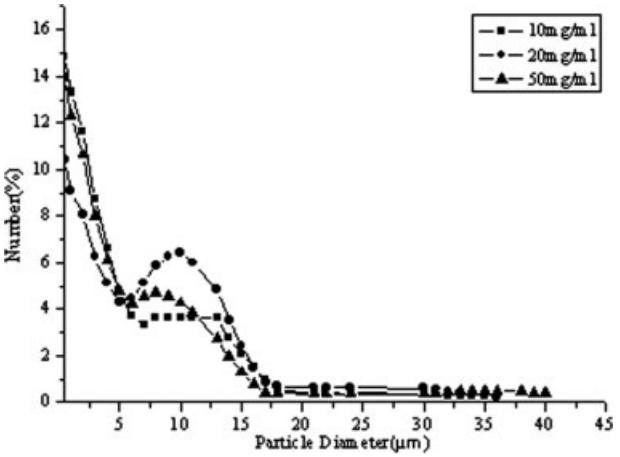

Figure 3 Effect of PVA concentration on particle size of microspheres.

as the external water phase to examine the effect of PVA concentration on the characteristics of the microspheres. The results are indicated in Table II (formulation E, C, and F) and Table III (formulation E, C, and F). As the PVA concentration increased, the diameter of microspheres slightly decreased (Table II and Fig. 3). It seems that a decrease in particle size could be achieved by increasing the concentration of PVA in the external water phase. A higher PVA concentration could increase the stability of emulsion droplets formed during homogenization because the increased viscosity of the external water phase would prevent emulsion droplets from coalescence, resulting in smaller emulsion droplets. These emulsion droplets gradually hardened to form microspheres as the solvent in the emulsion droplets continued to evaporate. Therefore, the size of the microparticles was relied on the size of the emulsion droplets formed during homogenization. It was observed that as its concentration increased, the encapsulation efficiency and the loading increased accordingly. But increase beyond an optimum value led to a gradual decrease. The maximum encapsulation efficiency and loading were obtained when 20 mg/mL PVA was used. This may have been due to the role of emulsifier. Below a critical concentration, the amount of emulsifier in the medium was not enough to stabilize all the microspheres and thus some of the drug was dissolved in the aqueous phase and lost before the evaporation of the solvent. On the other hand, when the emulsifier used was more than the critical level it coated all the surfaces excessively and might interact with available free drug during microcapsule formation resulting in its leakage into the aqueous phase.

Figure 4 shows the surface of microspheres prepared with different PVA concentrations in the external water phase. The lower PVA concentration (10 mg/mL) takes on more porous morphology, whereas the higher PVA concentration (20 mg/mL) shows less porous morphology. However, When the PVA concentration increases to 50 mg/mL, the surface of microspheres shows more porous than that of 20 mg/mL. This surprising phenomenon enabled

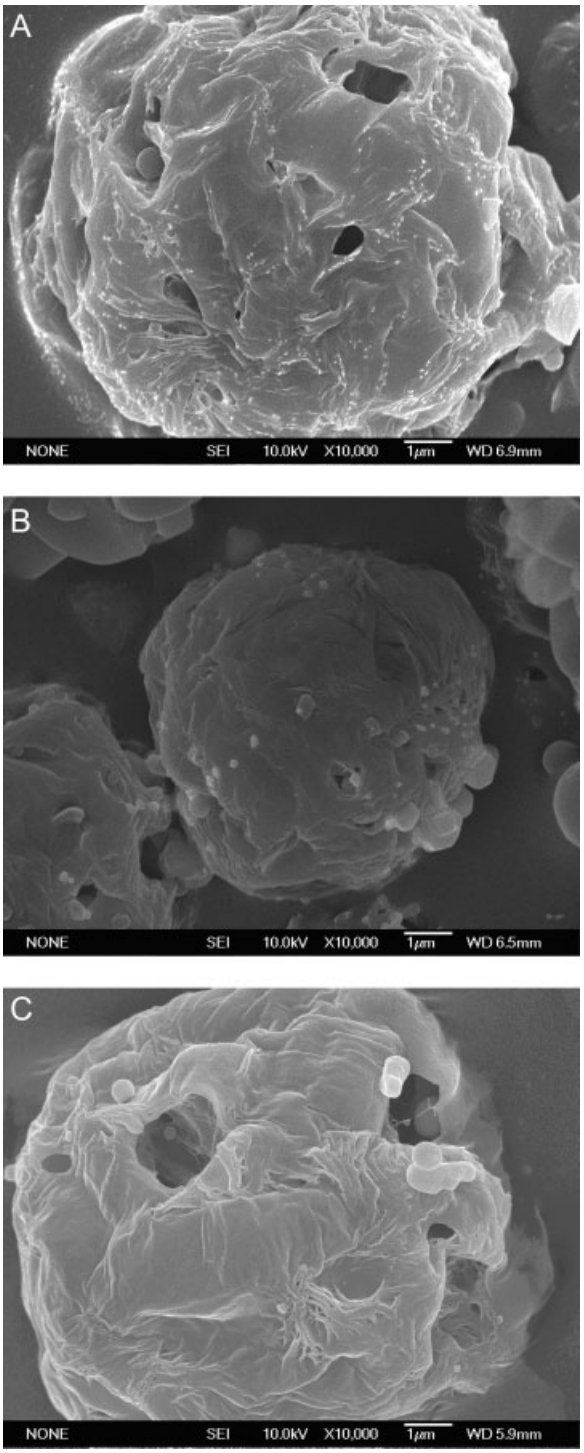

Figure 4 Surface of PHB microspheres prepared with different PVA concentrations. Caption: A, B, and C represent 10, 20, and 50 mg/mL PHB, respectively. Size of the bar is 1 μm.

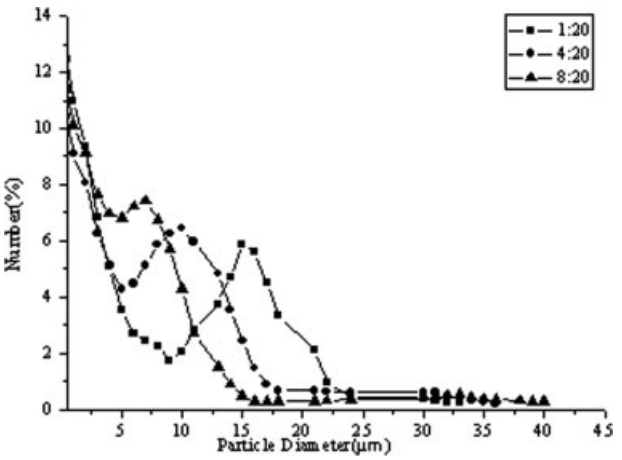

Figure 5 Effect of volume ratios (W1/O) of inner water phase/oil phase on particle size of microspheres.

us to predict that both lower concentration and higher concentration of emulsifier could not stabilize the microcapsules enough, only a proper value of emulsifier is needed.

Effect of volume ratio of inner water phase to oil phase (W1/O)

The volume ratio (W1/O) of inner water phase/oil phase also plays a critical role in the characteristics of the microspheres. Table II and Table III lists the properties of microspheres prepared with different volume ratios of inner water phase to oil phase. It was observed that the average size of microspheres decreased (Table II and Fig. 5) on increasing the volume ratios of inner water phase to oil phase. The change of size was perhaps due to the fact that the relatively higher viscous oil solution made the droplets break down into smaller droplets with difficulty. The decreased BSA encapsulation efficiency with an increase at volume ratios of water phase/oil phase might be due to the fact that the higher viscous oil solution could hinder the BSA in inner water phase diffusion to the external water phase, so the encapsulation efficiency of microspheres prepared with lower water phase/oil phase volume ratio was more than that of higher water phase/oil phase volume ratio.

Figure 6 illustrates the surface morphology with different volume ratios (W1/O) of inner water phase/oil phase. The surface of microspheres prepared with lower water phase/oil phase volume ratios is less porous and the higher water phase/oil phase volume ratios is more porous. A possible reason for this was that the aqueous phase droplets within microspheres which were prepared with higher water phase/oil phase volume ratio left more holes on the surface of the microspheres during the evaporation process.

Effect of volume ratio of primary emulsion to external water phase ((W1/O)/W2)

The effect of (W1/O)/W2 on the characteristics of the microspheres is shown in Table II (formulation I, J, C, and K) and Table III (formulation I, J, C, and

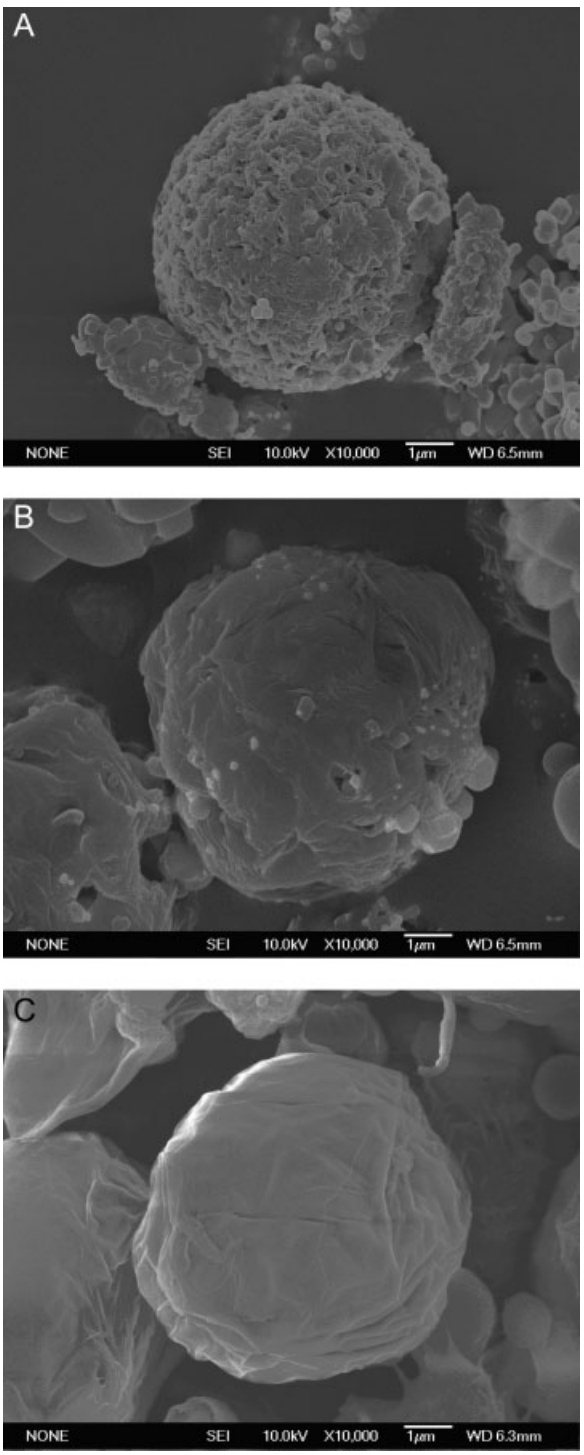

Figure 6 Surface of PHB microspheres prepared with different inner water phase/oil phase volume ratios. Caption: A, B, and C represent 8 : 20, 4 : 20, and 1 : 20, respectively. Size of the bar is 1 μm.

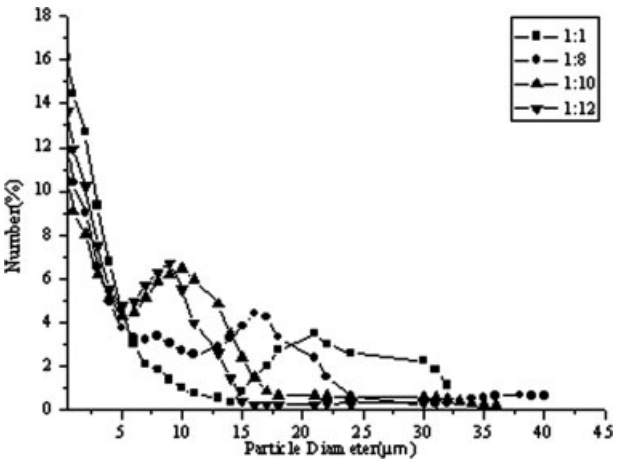

Figure 7 Effect of volume ratios of primary emulsion to external water phase on particle size of microspheres.

K). With the volume ratios increase of external water phase, the sizes of microspheres decrease (Table II and Fig. 7). The reason was perhaps that the viscous of double emulsion gradually decreased with the volume ratios increase of external water phase, then making the droplets more easily breaking down into small droplets. The encapsulation efficiency gradually decreases from 69.8% to 20.4% when the volume ratios of external water phase increases from 1 : 1 to 1 : 12. This might be that higher volume ratios of external water phase resulted in the higher osmotic pressure between inner water phase and external water phase; this could make the BSA in inner water phase largely leakage into the external water phase; so the encapsulation efficiency of microspheres prepared with higher volume ratios of external water phase was less than that of microspheres prepared with lower volume ratios of external water phase. As shown in Figure 8, when the volume ratio of external water phase gradually increases, the surfaces of corresponding microspheres take on more porous. The reason perhaps is that much more BSA of inner water phase diffused into the external water phase resulting in the porous structure.

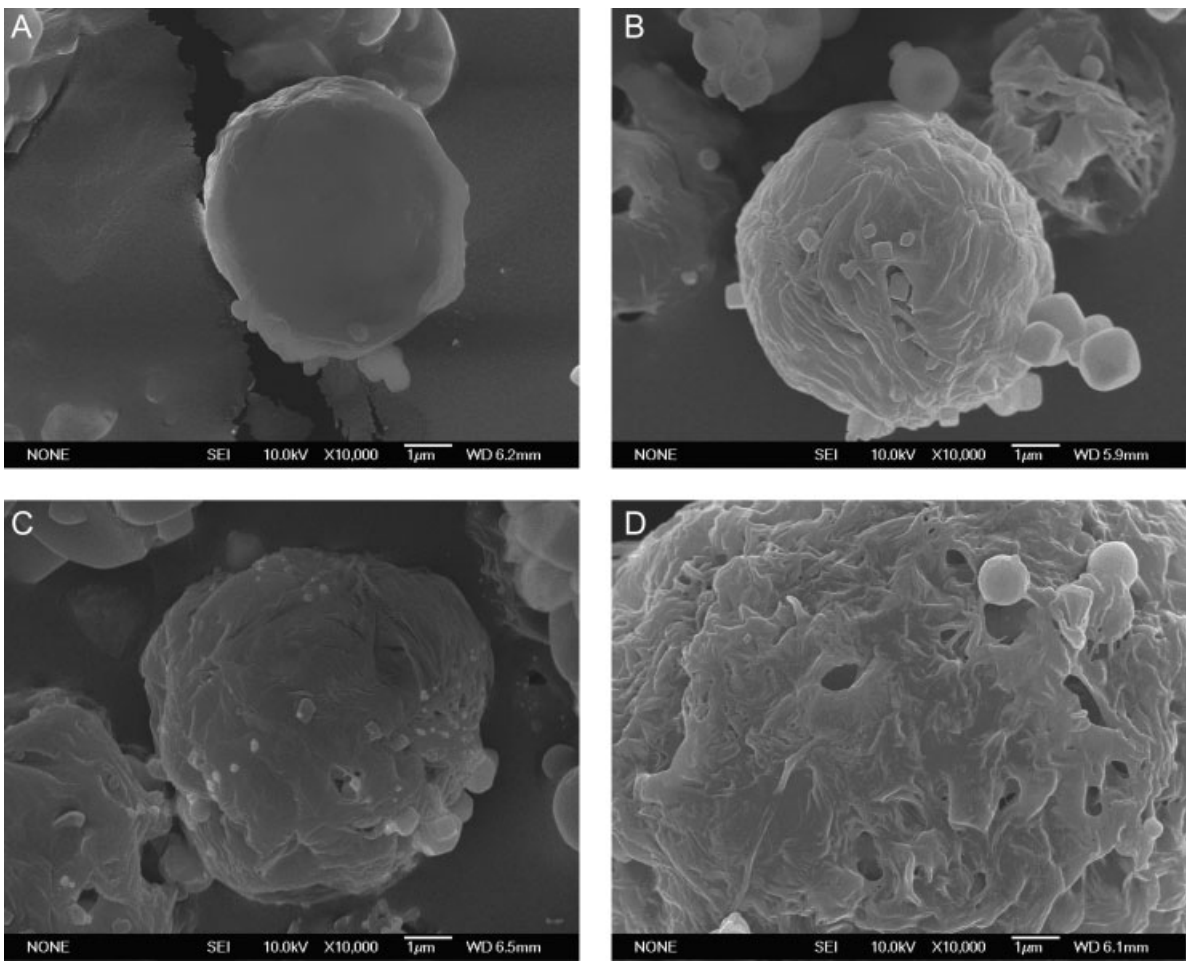

Figure 8 Surface of PHB microspheres prepared with different volume ratios of primary emulsion to external water phase. Caption: A, B, C, and D represent 1 : 1, 1 : 8, 1 : 10, and 1 : 12, respectively. Size of the bar is 1 μm.

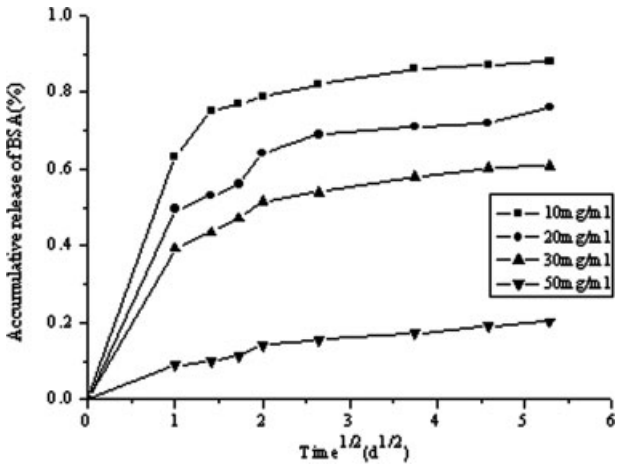

Figure 9 Effect of PHB concentration on release profiles of BSA.

In vitro release studies

PHB, as a biodegradable polymer, is generally degraded very slowly *in vitro*.[17] The rate of drug diffusion was substantially higher than that of polymer degradation, so the release profiles are more dependent on drug diffusion rather than on polymer degradation.

The diffusion of the drugs from spherical matrices can be expressed by the modified Higuchi equation as follows[18]:

$$\frac{M_t}{M_\infty} = \sqrt{\frac{D \times t}{\pi \times r^2}}$$

where M_t and M_∞ are the accumulative amounts of drug release at time t and infinity, respectively, D represents the diffusion coefficient of the drug, and r is related to the size of the microspheres. It indicates that the accumulative release amount is proportional to $t^{1/2}$.

Effect of the PHB concentration in the oil phase

Figure 9 shows the accumulative release (%) versus square root time curves of microspheres prepared at 10, 20, 30, and 50 mg/mL PHB concentrations, which reveal the release plots from 1 to day 28 are nearly in line after an initial burst of first day. So the whole release rate, over the period of day 1 to day 28, is controlled by the diffusion rate of drugs.

As the PHB concentration is increased from 10 to 50 mg/mL in the oil phase, the size of microspheres is increased from 7.8 to 15 μm, and their slope of release curves is decreased correspondingly; the experiment results are in agreement with the Higuchi equation. This could be illuminated as follows. The higher polymer concentration led to the larger size of microspheres which had relatively smaller surface-to-volume ratio than that of smaller size of microspheres. So, it was more difficult for the drug to diffuse through a longer diffusion distance.

Effect of PVA concentration in the external water phase

Release profiles of BSA-loaded microspheres prepared with PHB at 10, 20, and 50 mg/mL of PVA concentrations are shown in Figure 10. When the PVA concentration is increased from 10 mg/mL to 50 mg/mL, the size of microspheres is decreased from 12.5 to 11.5 μm. According to the Higuchi equation, the slope of release curve of the microspheres prepared with 10 mg/mL PVA in the external water phase should be lower than that of the microspheres prepared with 20 mg/mL and 50 mg/mL PVA in the external water phase. However, its slope was higher than that of them. This could account for the lower PVA concentration in the external water phase which could not stabilize the emulsion droplet enough, so the BSA distributed mainly toward the surface-associated area which led to a more rapid release.

Effect of volume ratio of inner water phase to oil phase (W1/O)

The microspheres are prepared using different W1/O. The BSA release profiles from these preparations are shown in Figure 11. The slope of the release curves is increased when the size of the microspheres is decreased from 16.3 to 6.9 μm, which are prepared with different W1/O. the Higuchi equation is fitted well by this results. It suggested that the relatively increased volume of inner water phase led to the formation of smaller emulsion droplets,

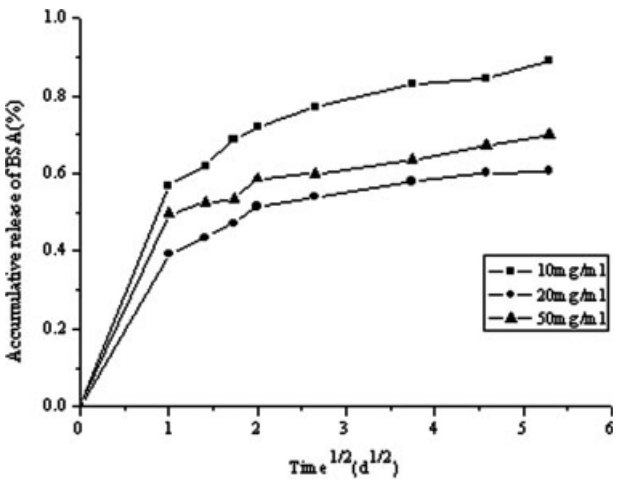

Figure 10 Effect of PVA concentration on release profiles of BSA.

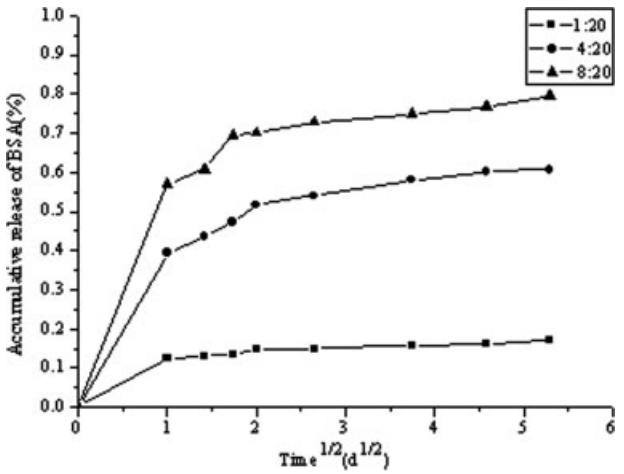

Figure 11 Effect of water phase/oil phase volume ratios on release profiles of BSA.

followed by the formation of smaller microspheres. Consequently, smaller mcirospheres with a greater surface area were formed, resulting in microspheres having more surface-bound BSA. Therefore, the BSA release from the smaller microspheres would be faster than that of the larger microspheres.

Effect of volume ratio of primary emulsion to external water phase [(W1/O)/W2]

Figure 12 shows the effect of different (W1/O)/W2 on the accumulative release of microspheres made with PHB. It is obvious that the slope of the release curves is increased on decreasing (W1/O)/W2 followed by size of the decreasing from 20.3 to 9.2 μm. It is confirmed that the BSA release behavior from microspheres is complied with the Higuchi equation. The reason was considered to be that the diffusive distance of BSA from the mircospheres would gradually become shorter on decreasing (W1/O)/W2.

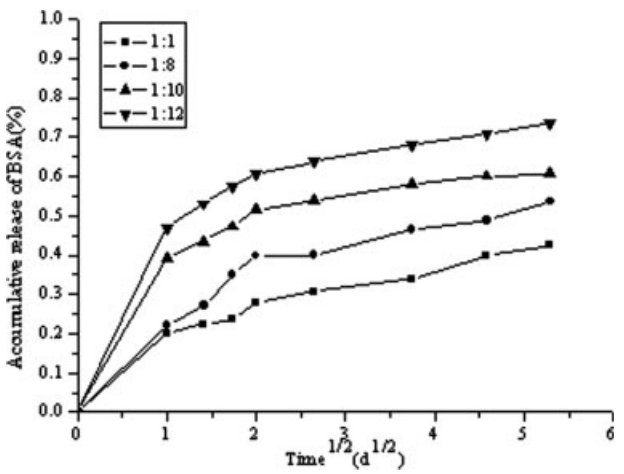

Figure 12 Effect of volume ratio of primary emulsion to external water phase on release profiles of BSA.

Interaction between BSA and polymer

In this work, the FTIR is used to explore the interactions between BSA and polymer. Figure 13 shows infrared spectrum of pristine BSA, pristine PHB microsphere, and BSA-loaded microspheres. The spectra of pristine BSA shows an amide carbonyl group at 1658.15 cm^{-1} and a carboxyl group at 1539.62 cm^{-1}. The pristine PHB microsphere illustrates a carbonyl group at 1718.01 cm^{-1} and an aether group at 1276.94 cm^{-1}. From the spectra of the BSA-loaded microsphere, it is observed that there are no significant changes in these bands. So, it is thought that strong chemical interactions between BSA and PHB were absent indicating the chemical stability of BSA in the microspheres. The spectrum is similar for all samples of microspheres produced with various preparation conditions and hence not all spectrum is shown.

The physical state of BSA inside the microspheres

DSC is always used to analyze the physical state of drugs encapsulated in the polymeric microspheres.[19] DSC thermograms of pristine BSA, BSA-loaded microspheres, and pristine PHB microspheres are displayed in Figure 14. Two characteristic features can be noticed. First, BSA shows a peak at 90.57°C due to melting, but in case of BSA-loaded microspheres, no characteristic peak is observed at 90.57°C, suggesting that BSA is molecularly dispersed in the mirospheres. Second, the pristine PHB microspheres shows peaks at 162.36°C and 171.36°C, however, this two melting peaks are displaced in the BSA-loaded microspheres. This phenomenon could

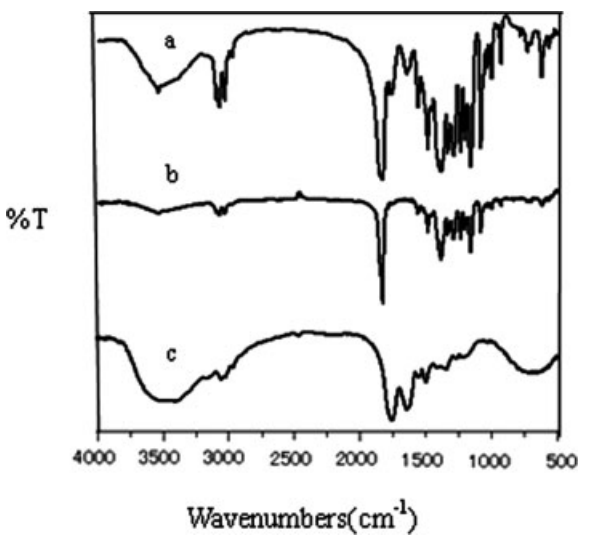

Figure 13 FTIR spectrum of (a) BSA-loaded microspheres; (b) pristine PHB microspheres; (c) pristine BSA. Caption: preparation conditions: PHB concentration: 30 mg/mL, PVA concentration: 20 mg/mL, W1/O: 1 : 5, BSA concentration: 30 mg/mL, and (W1/O)/W2: 1 : 10.

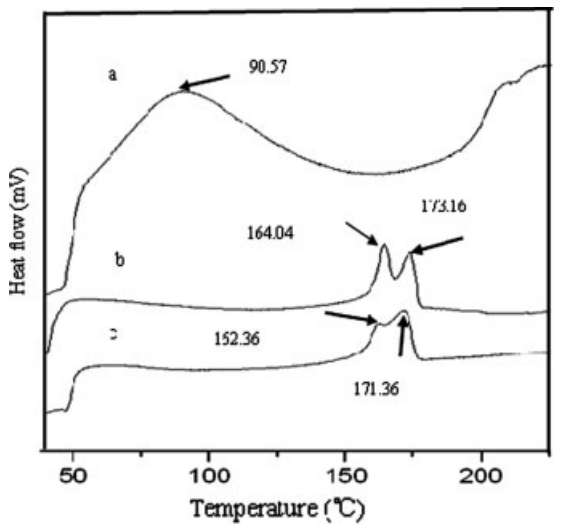

Figure 14 DSC thermograms of (a) pristine BSA; (b) BSA-loaded microspheres; (c) pristine PHB microspheres. Caption: Preparation conditions: PHB concentration: 30 mg/mL, PVA concentration: 20 mg/mL, W1/O: 1 : 5, BSA concentration: 30 mg/mL, and (W1/O)/W2: 1 : 10.

be ascribed to the BSA in the microspheres. The scans are similar for all samples of microspheres prepared using different preparation parameters and hence not all scans are shown.

CONCLUSIONS

PHB microspheres containing BSA were prepared successfully by the double emulsion technique and characterized by DSC, scanning electron microscopy, optical microscopy, and FTIR. DSC thermograms had confirmed the molecular distribution of the BSA molecules in the microspheres. SEM micrographs exhibited the spherical nature and different degree of porous structure due to the different preparation conditions. FTIR revealed that the chemical interaction between BSA and PHB was absent. The mean particle size of the microspheres ranged between 6.9 and 20.3 μm measured by optical microscopy, depending on the different preparation parameters. The maximum and minimum BSA encapsulation efficiency within the polymeric microspheres were 69.8 and 7.5%, respectively, varying with preparation conditions. The controlled release characteristics of the microspheres for BSA were investigated in pH 7.4 media. The initial BSA burst release from 8.9 to 63.1% followed by constant slow release for 28 days was observed for BSA from BSA-loaded microspheres and followed the Higuchi matrix model. So, the release behavior of microspheres showed the feasibility of BSA-loaded microspheres as controlled release devices. The biocompatibility, vitro degradation, vivo degradation, and bioactivity of drugs will be studied further in the future work.

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