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Electrospray encapsulation of water-soluble protein with polylactide Effects of formulations on morphology, encapsulation efficiency and release profile of particles☆

Yixiang Xu, Milford A. Hanna *

Industrial Agricultural Products Center and Department of Food Science & Technology, University of Nebraska, Lincoln, NE 68583-0730, USA

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Abstract

Bovine serum albumin (BSA)-loaded poly(lactide) (PLA) particles were prepared using an electrospraying technique, in which a sufficiently strong electric field was applied to overcome the surface tension of a droplet. A comprehensive investigation was conducted on the effects of independent variables organic/aqueous phase volume ratio and BSA/PLA weight ratio on the dependent variables viscosity, electrical conductivity, surface tension; the morphologies, sizes, and yields of particles; BSA encapsulation efficiency (EE); and in vitro release. An increase in the organic/aqueous phase ratio increased the viscosity and decreased the electrical conductivity of the emulsions, while the viscosity increased with BSA/PLA ratio. In general, spherical particles, with smooth surface and without visible pores, were observed. However, the spherical shape was lost as the organic/aqueous phase ratio decreased and the BSA/PLA ratio decreased. The particle sizes ranged from 0.84 ± 0.18 to 3.95 ± 0.51 μm and the yield was in the range of 64.3 ± 1.8 to $80.1 \pm 2.6\%$. EE of BSA was between 22.9 and 80.6%, and was increased with organic/aqueous phase ratio and decreased with increasing BSA/PLA ratio. In vitro release of BSA from the particles was reduced with increasing organic/aqueous phase ratio and was enhanced by the increase in the BSA/PLA ratio.

Keywords: Electrospray; Encapsulation; Poly(lactide); Bovine serum albumin; Encapsulation efficiency; Release rate

1. Introduction

Increasing attention has been paid on the encapsulation of biologically active ingredients into biodegradable polymers in the past two decades. This technique is applied widely in pharmaceutical, food, and agrochemical fields for improving stability of bioactive compounds, controlling release of drugs and reducing environmental pollution. Poly(lactide) (PLA) has been used as a coating material by many researchers because of its biodegradability, biocompatibility, high mechanical properties as well as its status of regulatory approval (Yang et al., 2001a,b). Different encapsulation techniques have been reported, mainly based on a two-step emulsification process like water-in-oil and the solid-in-oil-water (Weidenauer et al., 2003). Of these approaches, the double emulsion (water-in-oil-in-water) solvent extraction/evaporation technique is the most appropriate method to encapsulate hydrophilic substances into polymer matrices (Ogawa et al., 1988; Alex and Bodmeier, 1989; Langer, 1998). However, in all cases, the polydispersity of the particle size was relatively high, and a pressure homogenization device was used to prepare particles with a lower polydispersity (Lamprecht et al., 1999).

Recently, a simple and unique one-step technique (electrospraying) had been developed to encapsulate protein/drug-loaded particles (Amsden and Goosen, 1997; Loscertales et al., 2002; Kuo et al., 2004). Electrospraying is used commonly for ionization and characterization of protein and DNA in mass spectrometry or respiratory drug delivery (Tang and Gomez, 1994; Ijseaert et al., 2001). The principle of electrospraying is that the high electrical field applied stretches the liquid meniscus at the capillary tip, which subsequently deforms and breaks off (Yeo et al., 2004). A schematic diagram of the experimental

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* Corresponding author.
E-mail address: mhanna1@unl.edu (M.A. Hanna).

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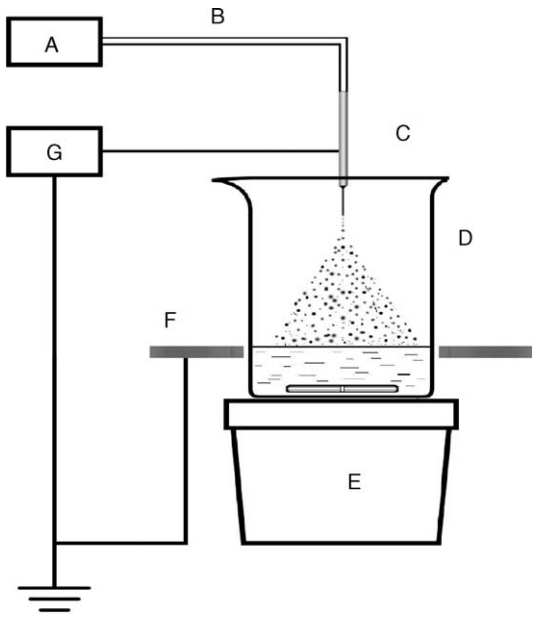

Fig. 1. Schematic representation of the electrospray apparatus: (A) pump, (B) feeding line, (C) 18-gauge, stainless steel needle, (D) 1000 ml beaker with water and magnetic stir bar, (E) magnetic stirrer, (F) copper collector ring (o.d. = 15 cm; i.d. = 11 cm), and (G) high voltage power supply.

equipment is shown in Fig. 1. A droplet forming on a needle tip will grow until its mass is large enough to escape the surface tension at the needle–droplet interface in the absence of an electric field (Sanders et al., 2003). When a high electric field is applied, the solution forms a conical meniscus. The meniscus further deforms and breaks into droplets with small particle sizes and narrow size distribution due to the pull of the electrostatic force. Coulombic repulsion between the highly charged droplets results in self-dispersion particles and no coalescence.

The electrospraying process is a complex process and is affected by many variables including the electrostatic field strength, needle diameter and the solution flow rate, physical properties, and concentration. A few researchers have reported on electrospraying encapsulation, but no information is available for PLA electrosprayed particles. The effects of processing and formulations on the morphology and particle size of bovine serum albumin (BSA)-loaded PLA particles were investigated by Xu et al. (in press). The objectives of this study were to comprehensively investigate the effects of the ratio of BSA to PLA and the organic to aqueous phase on the physiochemical properties of the resulting particles.

2. Materials and methods

2.1. Materials

Poly-L-lactide (M_W 175,000 Da), in the form of spherical granules of 2–4 mm, was purchased from Cargill Inc. (Minneapolis, MN). Bovine serum albumin (M_W 65,000 Da) was purchased from Sigma–Aldrich and used as provided. 1,2-Dichloroethane (1,2-DCE) and phosphate buffer saline (PBS, 0.067 M and pH 7.4) were of reagent grade, and were purchased from Fisher Scientific (Pittsburgh, PA).

2.2. Preparation of micro/nano particles

A PLA solution (3%, w/v) was prepared by dissolving 300 mg of PLA in 10 ml of 1,2-dichloroethane and stirring for 8 h at room temperature. Specified amounts of BSA previously dissolved in distilled water were mixed with PLA solutions and emulsified by sonication for 10 min. A 3^2 factorial design was used to prepare the BSA-loaded particles. Nine formulations based on the ratios of PLA/BSA and organic phase/aqueous phase are presented in Table 1. The emulsion was drawn into a 5 ml syringe attached with a blunt tip and 18-gauge metal needle. The syringe was placed in a syringe pump (Cole-Parmer 74900-00, Vernon Hills, IL) and a high voltage electrostatic system, with the range of 0–30 kV and a limiting current of 166 μA (Gamma High Voltage Research ES30P-5W/PRG, Ormond Beach, FL) was applied. The positive electrode of the electrostatic system was connected to the needle, while the negative electrode was placed in the collection solution 10 cm away from the needle tip. The solution was sprayed at a voltage of 12.5 kV and at a flow rate of 1 ml/h to a receiving beaker containing 200 ml of distilled water as the collection solution. The particles were separated from the collection solution by filtration and dried at room temperature.

2.3. Viscosity, electrical conductivity, and surface tension measurements

The viscosity measurements for PLA solution and PLA/BSA emulsions were performed with a Brookfield DV-II+

Table 1
The formulations of PLA/BSA emulsions and their physical properties

Formulation	Ratio of BSA/PLA (w/w)	Ratio of organic phase/aqueous phase (v/v)	Viscosity (mPa s)	Conductivity (μS/cm)	Surface tension (mN/m)
1	1:2	6.7:1	9.31^{d}	0.701^{a}	38.9^{a}
2	1:2	10:1	10.7^{b}	0.592^{b}	39.3^{a}
3	1:2	20:1	11.3^{a}	0.294^{c}	38.8^{a}
4	1:4	6.7:1	9.22^{d}	0.673^{a}	38.4^{a}
5	1:4	10:1	9.58^{d}	0.576^{b}	38.3^{a}
6	1:4	20:1	10.9^{b}	0.227^{c}	38.2^{a}
7	1:6	6.7:1	8.98^{e}	0.648^{ab}	38.5^{a}
8	1:6	10:1	9.27^{d}	0.532^{b}	38.6^{a}
9	1:6	20:1	10.3^{c}	0.205^{c}	38.9^{a}

Different superscript letters (a–e) means with same letter within a column indicate no significant ($p > 0.05$) difference by Ducan multiple range test.

programmable viscometer (Brookfield Engineering Lab. Inc., Middleboro, MA) at 25 °C with a SC-18 spindle speed of 200 rpm. The conductivity was determined using a digital conductivity meter with a gold dip cell (Model 2052, VWR Scientific, West Chester, PA). The cell was dipped into the container holding the solution and gently moved up and down a few times to dislodge any air bubbles. The measurement was recorded after the cell-equilibrated temperature to solution. Surface tension was measured by the ring method using a CSC-DuNouy tensiometer (Model 70545, CSC Scientific Inc., Fairfax, VA). The clean ring attached to the lever arm was placed into a container holding the solution whose surface tension was to be measured. The surface tension was the force of the pull exerted on the ring at the breaking point of the film.

2.4. Morphology, size, and structure characterization of particles

The morphology and size of BSA-loaded PLA particles were measured using scanning electron microscopy (SEM) at a voltage of 15 kV (Hitachi S-3000N, Tokyo, Japan). Before testing, the samples were mounted on the SEM stubs with double-sided adhesive tape and coated with platinum under vacuum to make the sample conductive. To calculate particle size, a random sampling of 100–150 individual particles was taken to minimize potential selection bias (Kuo et al., 2004). The size of the particle was calculated as the average of the shortest and longest dimensions.

2.5. Yield, protein load, and encapsulation efficiency

The yield was determined gravimetrically as the ratio between the mass of dried BSA-loaded particles to the total initial mass of polymer and BSA.

$$\text{Yield} = \frac{\text{mass of particles}}{\text{total mass of polymer and BSA}} \times 100\%$$

Loading capacity and encapsulation efficiency were evaluated by measuring the non-entrapped protein using the method of Xu and Du (2003). The amount of free BSA in collection solution was determined by UV spectrophotometry at 280 nm using collection solution of non-loaded particles as basic correction. The BSA loading capacity (LC) and encapsulation efficiency (EE) were calculated as:

$$\text{LC} = \frac{A - B}{C} \times 100$$

$$\text{EE} = \frac{A - B}{A} \times 100$$

where A was the total amount of BSA, B the free amount of BSA in collection solution, and C was the weight of the particles.

2.6. In vitro release

In vitro release measurement was performed using the method of Carrasquillo et al. (2001) with some modifications. BSA-loaded particles (10 mg) were suspended into 2 mL of PBS solution in a centrifuge tube containing 0.02% (w/w) sodium azide. The samples were incubated at 37 °C in a reciprocal water bath shaker (New Brunswick Scientific Co., Inc., Edison, NJ) operating at 100 rpm. At certain time intervals, the samples were ultra-centrifuged, and the supernatant was taken for test and replaced by the same amount of fresh PBS solution. The amount of BSA released from particles was analyzed by UV spectrophotometry at 280 nm. Total protein concentration values were used to construct cumulative release profile. The experiments were performed in triplicate.

2.7. Statistical analysis

A randomized complete block design (RCBD), with three blocks representing the replications, was used to prepare and characterize the particles. The diameters of the particles were analyzed as general linear models (GLM) using SAS v 8.0 statistical analysis software (SAS Institute Inc., Cary, NC). An analysis of variance (ANOVA) was employed to estimate the significance ($p < 0.05$) of the organic/aqueous phase volume ratio and the BSA/PLA weight ratio.

3. Results and discussion

3.1. The physical properties of PLA/BSA emulsions

The physical properties of the PLA/BSA emulsions played a critical role in determining morphology and size of particles, and release profiles during the electrospraying process. The viscosity, electrical conductivity, and surface tension of the emulsions are summarized in Table 1. Since the emulsions were non-Newtonian fluids, the viscosity was reported after stirring for 3 min. It can be seen that the viscosity of the emulsions generally decreased as organic/aqueous phase volume ratio decreased. For example, the viscosity of the emulsion decreased from 11.3 to 9.31 mPa s when the ratio was decreased from 20:1 to 6.7:1 and PLA/BSA weight ratio was 2:1. This was due to the fact that the emulsions were diluted with the addition of more water, resulting in a decrease in the viscosity. In addition, the viscosity of the emulsions increased as the BSA/PLA weight ratio increased from 1:6 to 1:2, which might result from the increase in total solid mass in the emulsions with increasing amounts of BSA.

Of the two factors, the organic/aqueous phase volume ratio was a key factor for the electrical conductivity of the emulsions. The changes in organic/aqueous volume ratio were found to have a significant ($p < 0.05$) effect on electrical conductivity. It can be seen that the electrical conductivity of the emulsions, for a fixed PLA/BSA ratio, increased by at least 2.4 times as the organic/aqueous phase volume ratio decreased from 20:1 to 6.7:1. Water is a polar substance and has a (high) electrical conductivity of 16.0 mS/cm, while 3% PLA in 1,2-DCE had a (low) electrical conductivity of 0.058 μS/cm. The addition of excess water to organic phase significantly increased the electrical conductivity of the resulting emulsions. On the other hand, BSA/PLA weight ratio had less effect

on the electrical conductivity. The electrical conductivity did not change considerably, having values of 0.592, 0.576, and 0.532 μS/cm, respectively, when BSA content increased from 14 to 33 wt% in a fixed aqueous/organic phase (e.g. 1:10). Both factors had no effects on the surface tension of the emulsions, having a constant value of approximately 38.6 mN/m.

3.2. Morphology, size, and yield of particles

A representative SEM micrograph of BSA-loaded PLA particles from formulation #6 is shown in Fig. 2. As can be seen, the majority of the particles were spherical with smooth surfaces and without visible pores. This may be attributable to the fact that the high boiling point (83.4 °C) of the 1,2-DCE slowed the evaporation rate during electrospraying, thus decreasing formation of pores. To further investigate the effects of the compositions on the surface morphologies of the particles, high magnification SEM micrographs from several formulations are given in Fig. 3. When the organic/aqueous phase volume ratio decreased from 20:1 to 6.7:1, with constant BSA/PLA weight ratio, the spherical shape of the particle was lost and the surface was wrinkled (Fig. 3A). This change was a consequence of the decrease in the viscosity of the emulsions. The viscous emulsion suppressed the shrinkage of the droplets during solvent evaporation. In addition, the particles appeared to shrink as the BSA/PLA weight ratio decreased from 1:2 (Fig. 3A) to 1:4 (Fig. 3C) when organic/aqueous phase volume ratio of 6.7:1, and from

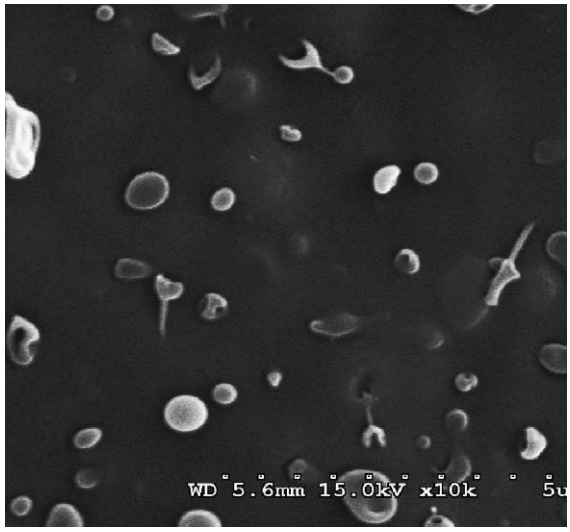

Fig. 2. SEM micrograph of BSA-loaded PLA particles from formulation #6.

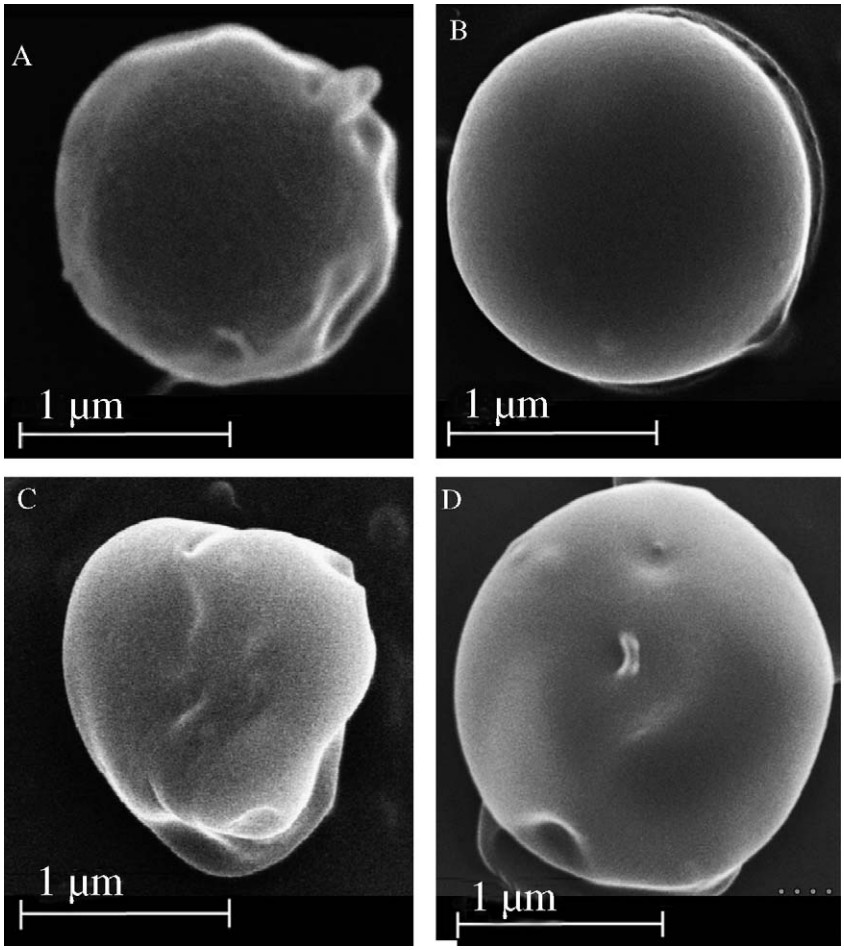

Fig. 3. SEM micrographs of BSA-loaded PLA particles at high magnification from: (A) formulation #1, (B) formulation #3, (C) formulation #4, and (D) formulation #9.

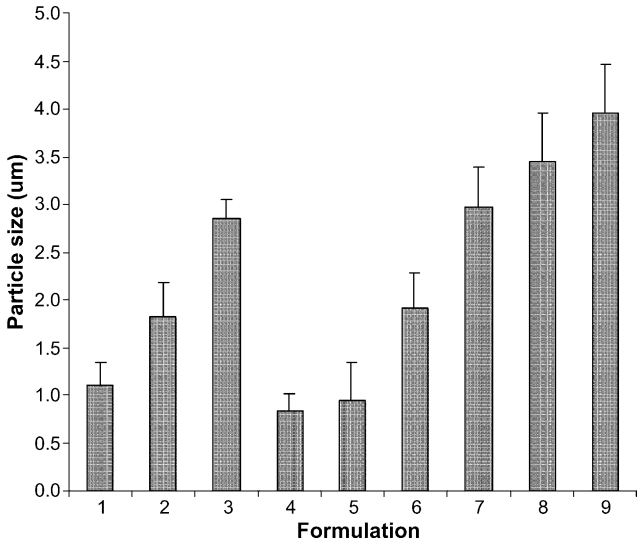

Fig. 4. Size of the particles prepared from different formulations.

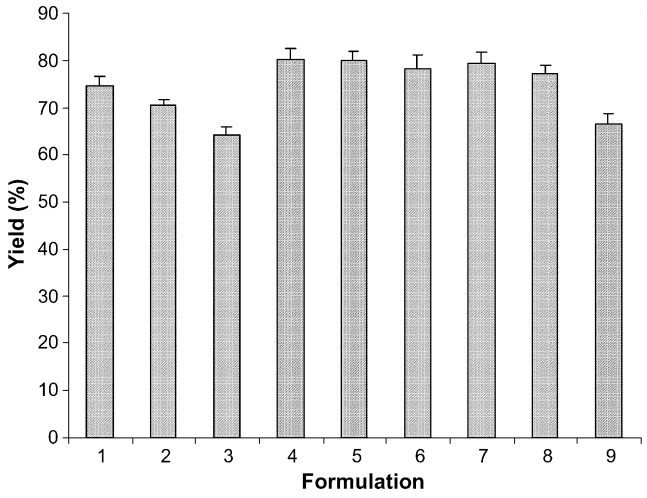

Fig. 5. Yield of the particles prepared from different formulations.

1:2 (Fig. 3B) to 1:6 (Fig. 3D) when organic/aqueous phase volume ratio of 20:1, respectively. In a diluted aqueous phase, more water droplets were trapped within the interior of spheres, and their evaporation during drying left empty spaces, resulting in shrinkage (Yang et al., 2001a,b). The shrinkage of the particle in Fig. 3C was more obvious than its counterpart in Fig. 3D, mainly because of the low PLA content and viscosity.

The particle sizes ranged from 0.84 ± 0.18 to 3.95 ± 0.51 μm (Fig. 4). The organic/aqueous volume phase ratio and BSA/PLA weight ratio had significant ($p < 0.001$) effects on particle size. However, the interaction between these two factors was negligible ($p = 0.109$) from AVOVA statistical results. This indicated that the changes in particle size with changes in organic/aqueous volume phase were not affected by BSA/PLA ratio, and vice versa. The particle size increased with the organic/aqueous phase ratio increasing from 6.7:1 to 20:1 at each BSA/PLA ratio level. The particle size, fabricated from the electrospraying process, was controlled mainly by the electrical conductivity and viscosity of the emulsions (Felder et al., 2003; Xu et al., in press). The viscosity increased and electrical conductivity decreased as the organic/aqueous phase increased from 6.7:1 to 20:1. It became more difficult for the viscous solutions with low electrical conductivities to be broken down into smaller droplets at the tip of the needle at the same electrical field. Moreover, the particle size first decreased, and then increased as the BSA/PLA weight ratio decreased from 1:2 to 1:6. The decrease in particle size with decreasing BSA amount arose from the decrease in viscosity caused by the total solid mass in the emulsion. The increase in particle size, with further decreasing BSA amount, might be due to low electrical conductivity.

The yield of particles ranged from 64.3 ± 1.8 to $80.1 \pm 2.6\%$ (Fig. 5). The changes in the organic/aqueous phase volume ratio and BSA/PLA weight ratio had no significant effects on the yield, although a slightly low yield was observed for BSA/PLA ratio of 1:2. The loss of solids resulted from attachment of particles to the glass vessel wall during electrospraying and on the filter paper during filtration.

3.3. BSA loading capacity and encapsulation efficiency

High BSA loading capacity was observed for all formulations, having a minimum value of $74.2 \pm 1.38\%$ for formulation #1 and a maximum value of $91.4 \pm 0.17\%$ for formulation #7 (Fig. 6). Each factor had a significant ($p < 0.001$) effect on the BSA loading capacity. However, there was no significant interaction between the factors. The BSA loading capacity was enhanced by 9.2, 9.6, and 6.8%, respectively, for BSA/PLA weight ratios of 1:2, 1:4, and 1:6, as the organic/aqueous phase volume ratio increased from 6.7:1 to 20:1. Increasing the BSA/PLA weight ratio from 1:6 to 1:2 dramatically decreased the BSA loading capacity for each organic/aqueous phase ratio.

BSA encapsulation efficiencies of 22.9–80.6% were significantly ($p < 0.001$) affected by the organic/aqueous volume ratio and BSA/PLA weight ratio (Fig. 7). Moreover, there was a strong ($p < 0.001$) interaction between these two factors, indicating the effect of organic/aqueous phase ratio on the BSA encapsulation efficiency was affected by BSA/PLA weight ratio, and vice versa. The encapsulation efficiency increased with increases in

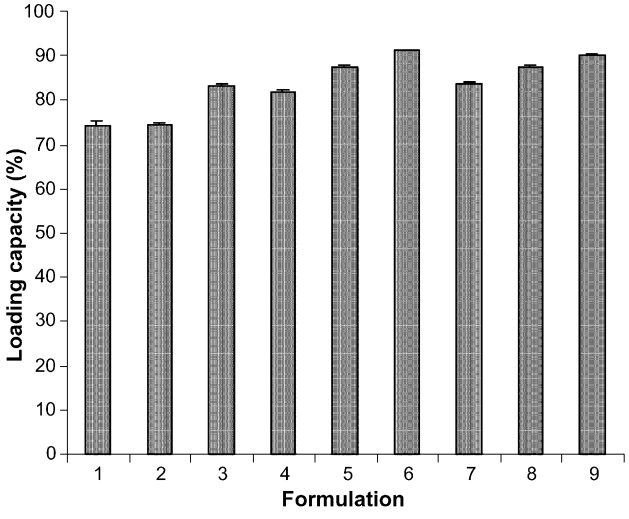

Fig. 6. BSA loading capacity of the particles from different formulations.

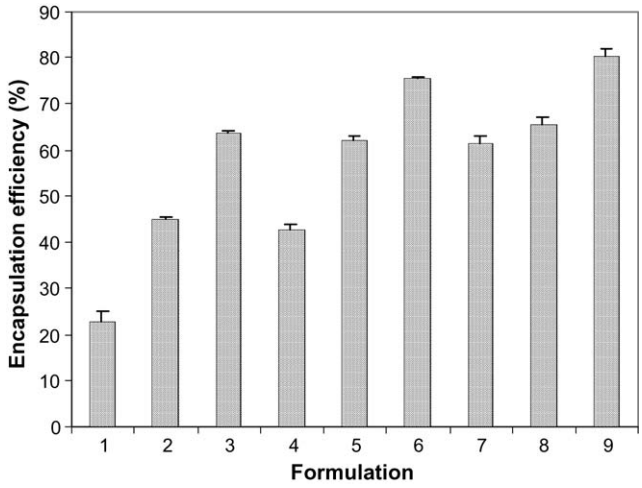

Fig. 7. BSA encapsulation efficiency of the particles from different formulations.

the organic/aqueous phase volume ratio from 6.7:1 to 20:1. This was due to the fact that the viscosity of the emulsion increased with increases in the organic phase ratio. High viscosity inhibited BSA droplet coalescence in the sprayed emulsion and the diffusion towards the aqueous collection bath, both limiting BSA losses (Freitas et al., 2004; Yang et al., 2001a,b). However, the increased rate in the encapsulation efficiency with increasing organic phase ratio was different, and depended on the BSA/PLA weight ratio, with values of 40.7, 32.8, and 19%, respectively, for BSA/PLA ratios of 1:2, 1:4, and 1:6. The effect of organic/aqueous ratio on the encapsulation efficiency for high-BSA emulsions was more obvious than that for low-BSA.

The increase in the BSA/PLA weight ratio from 1:6 to 1:2 markedly decreased the BSA encapsulation efficiency, especially for the emulsion with the lowest organic/aqueous phase ratio (6.7:1), a 38.6% decrease compared to 16.9% for its counterpart having the highest organic/aqueous phase ratio (20:1). This difference could be explained by the fact that the quantity of polymer in emulsion with the low organic/aqueous phase ratio was insufficient to cover the BSA completely (Benoit et al., 1999).

3.4. In vitro release of BSA from particles

In vitro release behaviors of BSA from selected formulations are shown in Fig. 8. In general, BSA release was time-dependent and consisted of three stages. A small initial burst, due to the desorption of protein molecules from the particle surface, occurred in the first 8 h, followed by a slow linear release between 8 and 50 h. This resulted from the diffusion of BSA dispersed in the polymer matrix. After 50 h, the release rate increased with time as a result of diffusion of the protein through the polymer walls, as well as the erosion of the polymers (Lamprecht et al., 2000).

The BSA release rates, in the first two stages, were similar for the particles having different BSA/PLA ratios (Fig. 8a). The initial burst was ~10% and increased to ~18% after the second stage release. However, noticeably different release profiles were observed for the particles in the third stage. The particles with

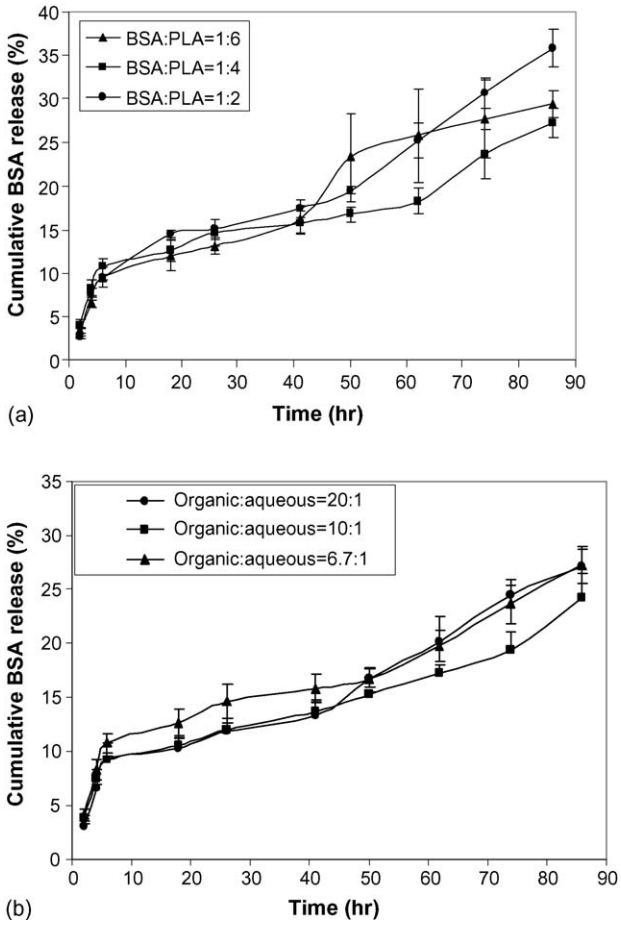

Fig. 8. BSA release profiles as functions of: (a) BSA/PLA weight ratios at a constant organic/aqueous phase volume ratio of 20:1 and (b) organic/aqueous phase volume ratios at a constant of BSA/PLA ratio of 1:4.

BSA/PLA ratio of 1:2 had the largest increase (83%) in the release rate between 50 and 90 h, followed by 49% for those with BSA/PLA of 1:4 and 25% for 1:6.

The particles with different organic/aqueous phase volumes ratios had similar initial burst releases of 10% (Fig. 8b). However, in the second stage, the release rate of particles with organic/aqueous phase ratio of 6.7:1 was significantly higher ($p < 0.05$) than those of its counterparts with organic/aqueous phase ratios of 10:1 and 20:1, indicating a slow release profile for high PLA concentration. This was consistent with the results of Yang et al. (2000). This could be explained by the fact that the particles fabricated with diluted solution had a thin wall; consequently, the diffusion path was short. The release rate of the particles with an organic/aqueous phase ratio of 20:1 increased dramatically, close to that having a ratio of 6.7:1 in the third stage. This was due to the degradation of the polymer walls.

4. Conclusions

The physicochemical properties of electrosprayed bovine serum albumin-loaded polylactide (PLA) particles were affected by the organic/aqueous phase volume ratio and the BSA/PLA

weight ratio, and physical properties of the emulsions. The viscosity of the BSA/PLA emulsions increased with increasing organic/aqueous phase and BSA/PLA ratios. The electrical conductivity of the emulsions increased with the increasing aqueous phase ratio. The spherical shapes of the particles were lost with decreasing organic phase ratio and BSA amount. The particle size increased with increasing organic phase ratio, while first decreasing and then increasing as the BSA/PLA weight ratio decreased from 1:2 to 1:6. The particles, from all formulations, had high yields (64.3 ± 1.8 to $80.1 \pm 2.6\%$), BSA loading capacities (74.2 ± 1.38 to $91.4 \pm 0.17\%$), and encapsulation efficiencies (maximum 80.6%). The encapsulation efficiency increased with increasing organic/aqueous phase ratio and decreased with increasing BSA/PLA ratio. High BSA/PLA ratio significantly increased the BSA release rate in the last stage, whereas a high organic/aqueous phase ratio slowed the BSA release.

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