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

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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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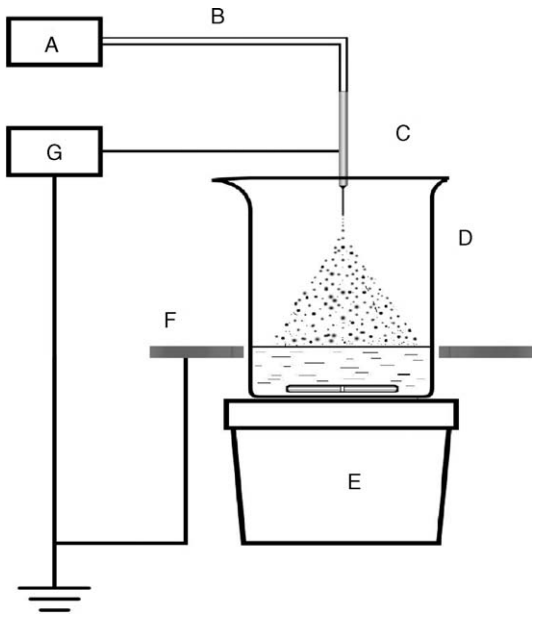

Fig. 1. Schematic representation of the electrospray apparatus: (A) pump, (B) feeding line, (C) 18-gauge, stainless steel needle, (D) 1000 ml beaker with water and magnetic stir bar, (E) magnetic stirrer, (F) copper collector ring (o.d. = 15 cm; i.d. = 11 cm), and (G) high voltage power supply.

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.