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RESEARCH ARTICLE

Fabrication and Characterization of Nanocapsules of PLGA Containing BSA Using Electrospray Technique

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ABSTRACT

Objective(s): Encapsulated pharmaceuticals are presently the object of comprehensive investigations in many research centers due to their increased therapeutic efficiency, bioavailability, and high dissolution rate. There are different procedures for encapsulation and choice of procedure influences the size of particles for intended applications.

Methods: In this study, Nanocapsules of Poly-Lactic-co-Glycolic Acid (PLGA) containing Bovine Serum Albumin (BSA) at ratios of 0.25/0.25, 0.4/0.1 and 0.45/0.05 were fabricated by electrospraying method. Also, the effect of some parameters in electrospraying was evaluated, including PLGA concentration, voltage and flow rate on the morphology and size of particles.

Results: BSA loaded PLGA Nanocapsules were successfully prepared by using electrospraying technique. The formation of capsules was confirmed by TEM. SEM results of the samples showed that decreasing the flow rate and increasing voltage decreased the average size of nanocapsules and led to producing the capsules with a size in the range of 85-260 nm. The presence of the drug in nanocapsules was confirmed by DSC results. Drug release test showed that about 90% of BSA had been released during 24 h.

Conclusions: PLGA nanocapsules containing therapeutic proteins were produced by the electrospraying technique under different operation parameters and physical properties.

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INTRODUCTION

Encapsulation of pharmaceutical materials was got widespread attention owing to bioavailability, therapeutic efficiency, and high dissolution rate. Several methods such as wet/ dry milling, solvent evaporation from emulsions, precipitation and spray-drying have been employed to produce small particles and many of them used in drug formulation [1-5]. However these particles generating techniques were used successfully, but may not be appropriate for some compounds or applications [3, 6]. Low encapsulation efficiency or tiresome separation procedure of particles from aqueous phase constitute the main deficiencies of these techniques [7]. On the other hand, these techniques are not suitable for the production of porous or hollow particles, composites, non-spherical particles and encapsulated materials [8]. Peptides and proteins gain great importance as pharmaceuticals in most cases but when given orally they are not stable and hence not effective [9]. Meanwhile, Proteins are prone to denaturation/ degradation, covalent/ non-covalent aggregation and non-specific adsorption on the polymer surface during the production process and likewise

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Table 1. Physical properties of PLGA/BSA solutions

Samples	%BSA (w/v)	%PLGA (w/v)	Viscosity (cp)
S_1	0.50	-	10
S_2	0.25	0.25	20
S_3	0.10	0.40	30
S_4	0.05	0.45	35
S_5	-	0.50	36

the release period. Instability of process, which reduces the biological activity of microencapsulated proteins, leads to a direct effect on the kinetics of drug release from polymer micro particles [10].

A suitable method for producing nano and micro particles of bioactive molecules is electrospray which is liquid atomization techniques using electrostatic forces [11].

Due to being biodegradable and biocompatible, Poly-lactic-co-glycolic acid (PLGA) has found biomedical applications such as drug delivery systems [12]. PLGA undergoes hydrolysis within the body into glycolic acid and lactic acid. Production of PLGA microspheres containing celecoxib through electrospraying explained in a paper by Bohr et al [13]. Recently, Zhu et al [14] fabricated inhalable oridonin-loaded PLGA porous microspheres using the electrospraying technique.

Bovine serum albumin (BSA) with high molecular weight has numerous biochemical applications. Also, BSA is a globular protein that is soluble in water [15]. Hence BSA was chosen as a model drug in the present study. Electrospraying of BSA studied in a paper by Pareta et al [16]. Since PLGA is a biodegradable polymer, by encapsulating the BSA in this polymer, PLGA will become as drug carrier and due to the protection of BSA from degradation and control release profile, BSA release occurs slowly and thereby reduces the risk of local overdose.

To our best knowledge encapsulation of BSA in PLGA have never been prepared using electrospraying so in the present study, BSA is encapsulated with PLGA, using an electrospray technique that in which a sufficiently strong electric field is applied to overcome the surface tension of a droplet and producing small particles. In addition, the effect of some parameters in electrospraying namely, PLGA concentration, the viscosity of PLGA solutions and PLGA/ BSA emulsions, voltage and flow rate on the morphology and size of particles are also studied.

MATERIALS AND METHODS

Materials

BSA (>96%) and PLGA (PLGA; 50:50, Mw = 24000-38000 KDa) was purchased from Sigma-Aldrich. Acetone (99.9% HPLC grade), acetic acid (99.9% HPLC grade) and ethanol (99.9% HPLC grade) were purchased from Merck.

Preparation of electrospraying solutions

Spraying solutions were prepared by adding PLGA to acetone and BSA to ethanol/acetic acid with ratio of 24ml/1ml at room temperature. Several spraying solutions were made with different concentrations of PLGA and BSA (Table. 1). The prepared solutions were filtered off and mechanically stirred for obtaining clear solutions for electrospinning. The spraying solution viscosity was measured by using a Viscometer (Brookfield DVII Viscometer, USA) with spindle speed of 30 rpm at ambient temperature. Before measurement by using either ethanol or distilled water where values are known, the Viscometer was calibrated.

Nanocapsules preparation

The spraying system consisted of voltage power source (Gamma high voltage research, USA), syringe and needle, dosing pump (New Eva pump inc, USA) and collector. The syringe (1 ml) was loaded with the solutions containing dissolved polymer and drug then connected to the dosing pump. It is sprayed after applying a voltage to generate capsules. The capsules generated from liquid jet were collected on an aluminum foil. Electrospraying factors such as flow rate (0.1 and 0.2 µl/min), collector distance (10, 15 and 20 cm), applied voltage (10, 15 and 20 kV) and PLGA/BSA

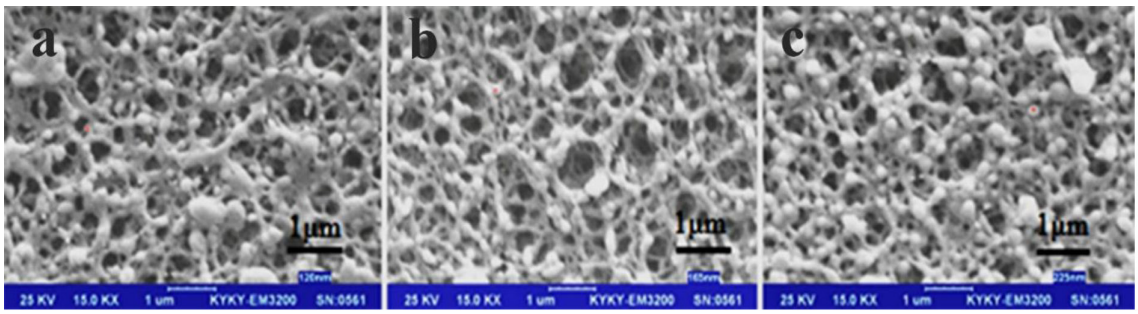

Fig. 1. SEM images of nanocapsules produced at voltage=10 kV, nozzle–collector distance=10 cm and flow rate 0.2 µl/ min but different PLGA/BSA ratios: (a) 0.25/0.25 w/v; (b) 0.4/0.1 w/v; (c) 0.45/0.05 w/v.

ratios (0.25/0.25, 0.4/0.1 and 0.45/0.05) were used to partly control capsule formation.

Morphology, size and characterization of nanocapsules

The morphology and size of capsules were investigated by scanning electronic microscopy (SEM) (Model XL 30, Philips, Netherland). For each sample, the diameter of the capsule was measured from the SEM images using Digimizer software. Before testing, the samples were precoated with an ultra thin gold layer through using a sputter coater for 90s (BAL-TEC co. Model sc Doos, Switzerland). The internal structure was characterized by transmission electron microscope (TEM) EM-10 (Model, Zeiss-West Germany).

UV spectroscopy

A UV spectrophotometer (Model cintra 10) was used to measure UV absorption peak of BSA at 268 nm by using appropriate calibration and blanking procedures before measurements. In determining drug release, the amount of dried capsule samples were weighted and suspended in 10 ml PBS (pH=7.4), mixed for 24 h under continuous stirring. The buffer solution was kept constant at 37 °C. The precipitated capsules were taken and resuspended in 10ml fresh PBS and placed back in the stirrer. In discrete (1 h) time the volume of samples was centrifuged for 20 min at 4000 rpm, the supernatant was extracted and its release was estimated at 268 nm with an ultraviolet detector.

Differential scanning calorimetry

Thermal properties of capsules were studied by differential scanning calorimetry (DSC) (Bähr thermanalyzer 302, Germany). Measurements of pure polymer and drug loaded polymer solutions carried out. Approximately (5 mg) samples were loaded onto standard aluminum pans and purged with dry nitrogen. The samples were heated from room temperature to 250 °C with scan rate 10 °C/ min.

RESULTS AND DISCUSSION

Capsules formation

Table 1 shows the physical property of solutions in this study. Polymer concentration played an important role in the ability of electrospraying. If the polymer solution is too concentrated, solution viscosity increases, hence it is difficult to control the solution flow rate through the capillary. Therefore, an optimum range of polymer concentration for solution capability to electrospraying is necessary. Various polymer concentrations could be effective on the size and shapes of a capsule and it is obvious that the capsule size could decrease with decreasing polymer concentration [17]. The surface of

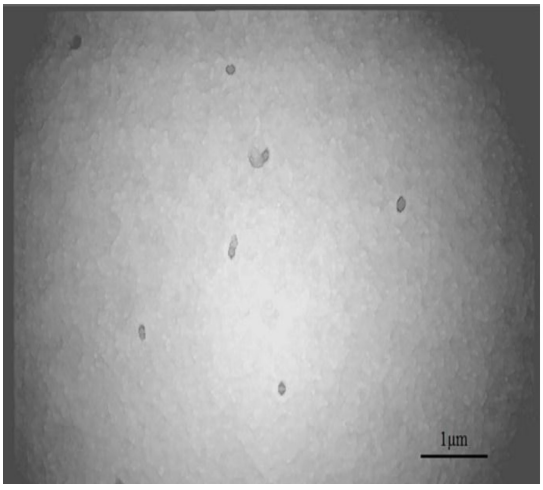

Fig. 2. TEM image of PLGA/BSA nanocapsules.

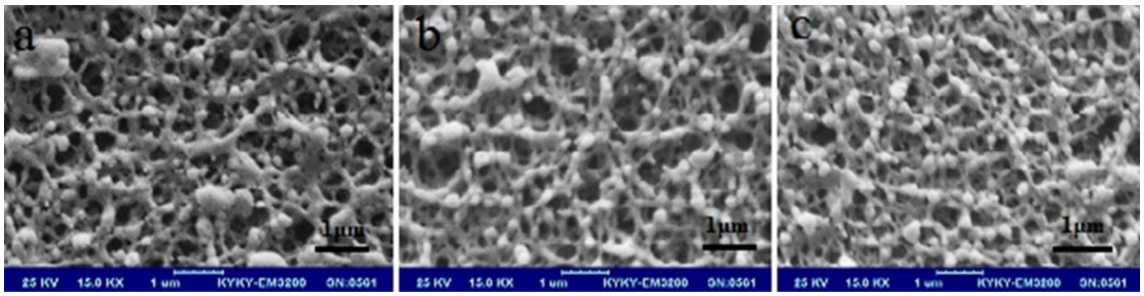

Fig. 3. SEM images of nanocapsules produced with flow rate= 0.2 µl/min, PLGA/BSA ratio= 0.25/0.25 w/v (a) voltage =10kV, nozzle–collector distance =10cm; (b) voltage =15kV, nozzle–collector distance =15cm; (c) voltage =20kV, nozzle–collector distance =20cm

PLGA capsule maybe change, when polymer concentration decreasing, from smooth surfaces to harsh wavy surface. Also, the capsule could change from regular sphere shape to biconcave irregular with decreasing polymer solution concentration.

The capsules at different concentration are shown in Fig. 1. Capsules were not observed for 0.25% w/v concentration (Fig. 1a) especially in lower flow rates (Fig. 4c) since it was difficult for PLGA to form a capsule from a diluted solution. Capsules were obtained with the PLGA concentration of 0.4% w/v (Fig. 1b) in which some of the capsules were shrunk during solvent evaporation. Fully capsules were produced at 0.45% w/v PLGA (Fig. 1c) but the size of nanocapsules increased from 120 nm to 225 nm. This could be attributed to the viscosity of spraying solution that is consistent with the results obtained by Jayasinghe and Edirisinghe [18].

A TEM image of PLGA/BSA nanocapsules is shown in Fig. 2. The image clearly shows that encapsulation has successfully been achieved; where BSA is the core of capsules with PLGA is the shell, indicating that capsules are consist of two-phase (biphasic). Consequently, it can be stated that BSA is located right inside the PLGA capsules.

Effect of the applied voltage and collector distance from nozzle

Applied voltage and the distance between capillary tip and collector have the same considerable influence, because the electric field strength is measured by these parameters. Voltage

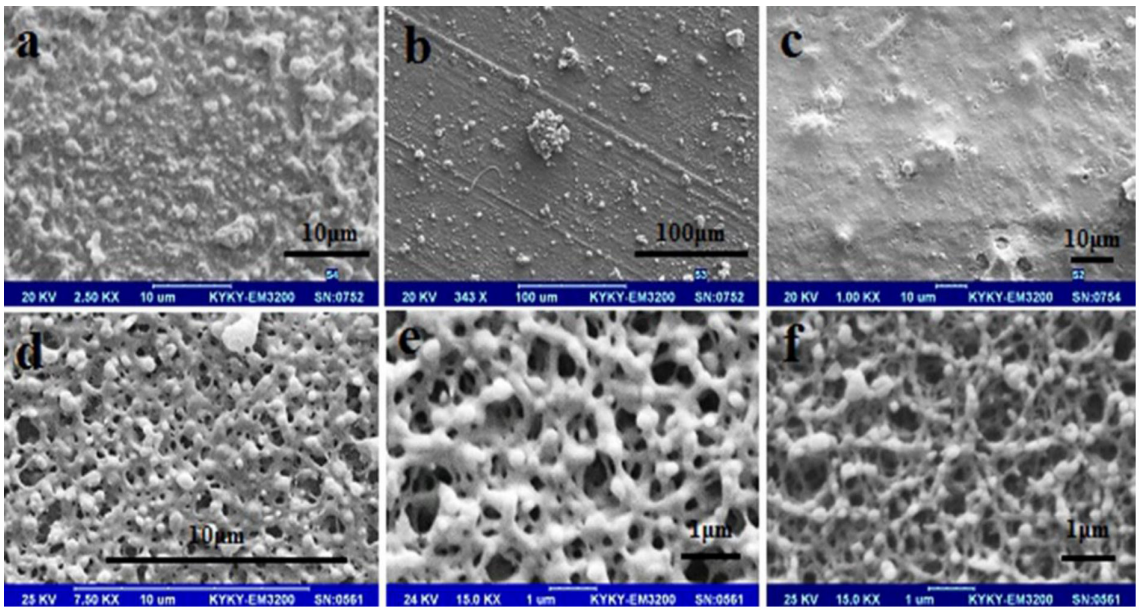

Fig. 4. SEM images of PLGA/BSA nanocapsules at voltage=10kV, nozzle–collector distance=10cm, flow rate 0.1 µl/ min: (a) PLGA/BSA ratio= 0.45/0.05 w/v (b) PLGA/BSA ratio= 0.4/0.1 w/v (c) PLGA/BSA ratio= 0.25/0.25 w/v. And flow rate 0.2 µl/ min: (d) PLGA/BSA ratio= 0.45/0.05 w/v (e) PLGA/BSA ratio= 0.4/0.1 w/v (f) PLGA/BSA ratio= 0.25/0.25 w/v.

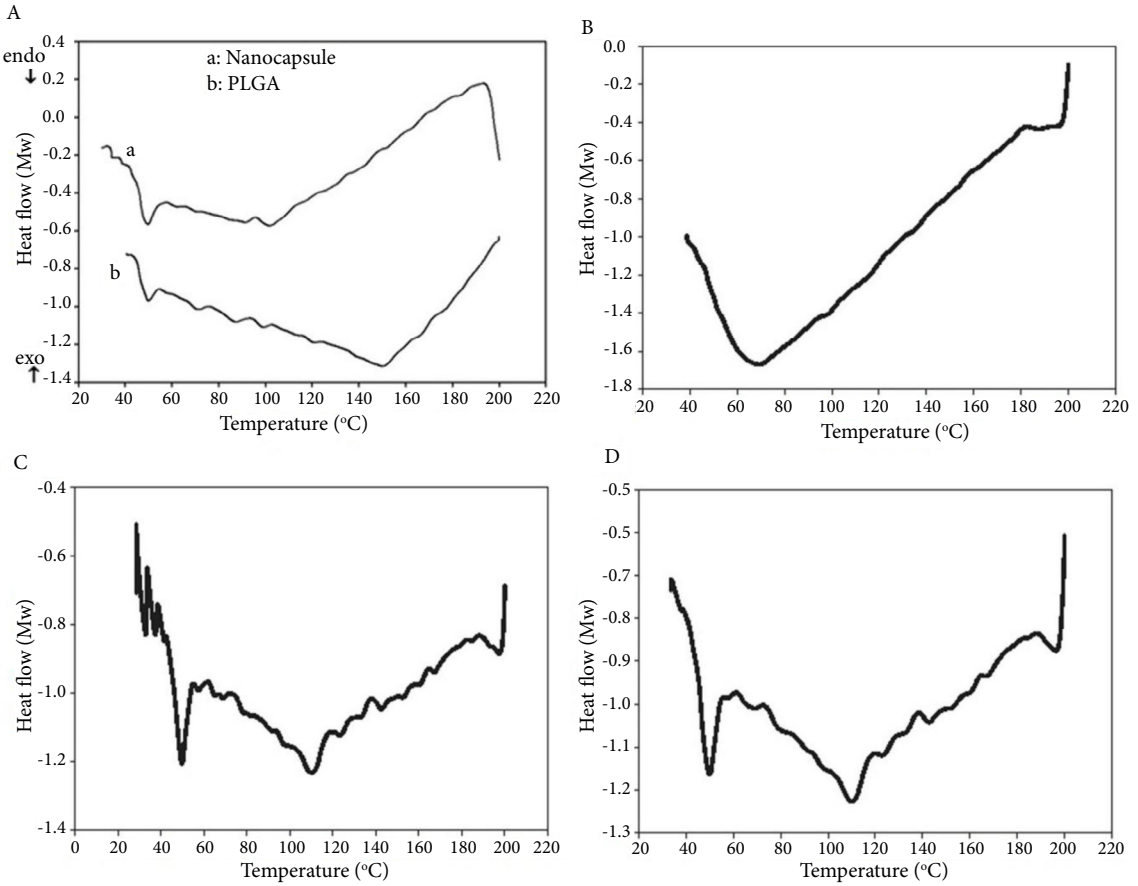

Fig. 5. DSC thermograms of (A) pure PLGA and nanocapsule of PLGA; (B) pure BSA; (C) nanocapsule of PLGA/BSA, 0.25/0.25 w/v (D) nanocapsule of PLGA/BSA, 0.45/0.05 w/v.

controls the morphology and size of the capsules. Three voltage levels (10 kV, 15 kV and 20 kV) were chosen and their influence on the morphology of capsules were investigated while holding the other condition constant (flow rate of 0.2 µl/ min).

Fig. 3 demonstrates the variation of the average size of nanocapsules at an applied voltage (10kV, 15kV and 20 kV). As can be seen, increasing the voltage from 10 kV to 20kV leads to decreasing in nanocapsules diameters from 185 nm to 85 nm. In fact, at higher voltages, overcoming surface tension of the solution becomes easier, which leads to the formation of smaller nanocapsules by electrospraying. The results agree with other studies [19].

Effect of the flow rate

The previous studies showed that there is a relationship between capsule diameter and flow rate [20]. Electrospraying of polymer solution achieved under two different flow rates 0.1 and 0.2 µl/min (Fig. 4). As seen from the Fig, by changing the flow rate from 0.2 µl/min (Fig. 4d-f)) to 0.1µl/ min (Fig. 4a-c) capsules size become smaller. This can be explained by the fact that the uneven spread of solution at the tip of the capillary caused by the high flow rate that results in uncontrolled electrospraying of large capsules. Although the capsule morphology seemed to be smoother when the polymer solution flow rate was high. Higher flow rates lead to bigger capsule sizes deposited in the collector. In fact, an increase of flow rates produces bigger droplets at the tip of the needle.

DSC analysis

DSC studies were performed for investigating the nature and intermoleucolar interactions of the encapsulated BSA and PLGA (Fig. 5). The pure BSA (Fig. 5B) shows an endothermic peak of melting point at 68 °C and a wide endothermic

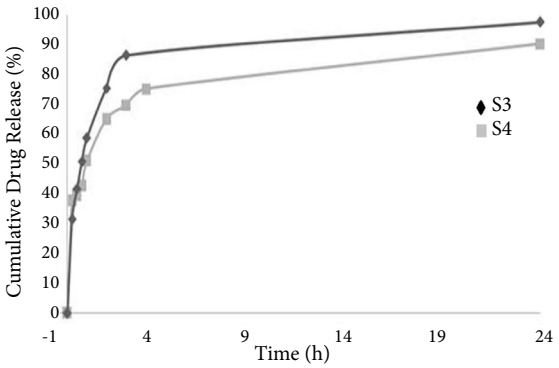

Fig. 6. Release of BSA from nanocapsule of PLGA/BSA with different ratio, (S3) 0.4/0.1 w/v, (S4) 0.45/0.05 w/v.

peak around 200 °C with an onset at 180 °C . The DSC curve of pure PLGA shows an endothermic melting peak at 154 °C and an endothermic peak at 50 °C corresponding to its glass transition temperature (Tg) but this peaks shifted down to lower temperature in nanocapsule (47 °C and 105 °C) as shown in Fig. 5A, this is attributed to the rearrangement of molecular during encapsulation. Sample PLGA/BSA at ratio 0.25/0.25 w/v (Fig. 5C) and 0.45/0.05 w/v (Fig. 5D) shows three endothermic peaks, two peaks at 47 °C and 105 °C for PLGA and another peak at 200 °C for BSA which confirms the presence of drug in nanocapsules. According to Fig. 5, BSA endothermic peak at 68 °C were not present in PLGA/BSA nanocapsules (Fig. 5C-D) which shows that BSA existed in an amorphous or disordered crystalline phase in nanocapsules [21]. Furthermore, the total peak area became narrow in the case of nanocapsules containing BSA as compared to the sample without BSA, which can be attributed to the interaction between PLGA and BSA in nanocapsules. Moreover, the glass transition temperature of polymer employed in capsulated BSA was not influenced obviously by the electrospray process.

Drug release studies

Fig. 6 shows the release of drugs from nanocapsules of PLGA/BSA with different ratio. As can be seen, rapid release of the drug in the first minutes could be attributed to the presence of freely available drug on the surface of nanocapsules without any linkage. Sample S3 is fastest and sample S4 is the slowest to release of drugs. According to Pinon-segundo finding, [22] by increasing the drug content in matrix observed faster release of drugs due to a higher amount of porosity that created in the matrix

The drug release was slowed over the time and according to the Fig. 6 after 24 h at 37°C temperature, 90% of the total amount of drug content released in buffer phosphate. The extreme decrease in drug release between 40-50% for both samples (S3 and S4) shows the large amount of bonding between BSA and nanocapsules.

CONCLUSIONS

Nanocapsules of PLGA containing BSA with controllable morphology and size were successfully produced by electrospray method under different operation parameters and physical properties. Capsules size increased with increasing PLGA concentrations in solutions and capsules could change from sphere to biconcave to the irregular shape with decreasing polymer solution concentration. Investigations showed that the increase in voltage at a constant flow rate reduces the size of nanocapsules but ultimately lead to the production of the fiber. When the flow rate increases, the capsules size increases while their shape became smoother. TEM micrographs indicated that the two-phase core and shell is quite visible. Studies showed the presence of BSA in the capsule and 90% of drug of nanocapsules was gradually released within 24 h.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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