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RESEARCH PAPER

Emulsion Spray-Drying for the Preparation of Albumin-Loaded PLGA Microspheres

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ABSTRACT

The purpose of this work was to study the encapsulation of bovine serum albumin (BSA) in polylactide-co-glycolide (PLGA) microspheres using an emulsion/spray-drying method. Albumin was dissolved in an aqueous phase (w) in the presence of surfactant and emulsified in an organic phase containing the polymer (o). To stabilize the emulsion, different types of surfactant (Pluronic® F68, Pluronic F127, sodium oleate, dioctylsulfosuccinate) were added to the aqueous phase. The w/o emulsion was spray-dried to obtain BSA-loaded PLGA microspheres. The effect of type of surfactant on microsphere characteristics was evaluated. The microspheres were characterized for their morphology by scanning electron microscopy (SEM) and granulometric analysis; drug content determination and in vitro dissolution tests were performed. Results showed that the emulsion/spray-drying method is suitable for obtaining small microparticles (2–5 μm) characterized by high drug payloads (70%–80% encapsulation efficiency). The type of surfactant affects the microsphere shape and BSA release behavior.

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INTRODUCTION

The encapsulation of peptides and proteins into biodegradable microspheres is an interesting topic of study currently for the formulation of controlled-release drug delivery systems for vaccines and hormones for either parenteral and oral administration (1). Due to its excellent properties in terms of biodegradation and biocompatibility, polylactide-*co*-glycolide (PLGA) represents the most studied biopolymer as a drug carrier.

The loading of peptides and proteins into polymeric micromatrices made of PLGA represents a problem from a technological point of view because they are hydrophilic, high molecular weight macromolecules; moreover, they are easily damaged by the various environments and solvents necessary for the manufacture of microspheres. The most used methods for the microencapsulation of proteins are the water/oil/water (w/o/w) double-emulsification and the oil/water (o/w) single-emulsification techniques, followed by solvent evaporation and/or extraction (2). Both methods involve intimate contact of the protein with an organic solvent like methylene chloride or chloroform for long periods of time (2 h or more) and heating. These conditions can be detrimental to protein stability; moreover, loss of some protein can occur in the outer aqueous phase. The microspheres obtained by these methods very often show high burst effect, up to 40% of the total encapsulated protein released in the first 24 h, due to the protein present on microsphere surface.

Besides these methods, a polymer phase separation method, which does not involve aqueous phases, has been used to encapsulate protein drugs into PLGA microspheres (3). This technique is carried out at room temperature, and it offers the advantages of not exposing the drug to heat or permitting its partitioning in an aqueous phase. Nevertheless, high residual solvent concentrations have been found in the resulting microspheres (4).

Recently, spray-drying has gained more and more importance as the preparation method of microparticulate drug delivery systems made of different kinds of polymers (e.g., polyesters, polymethacrylates, celluloses) and loaded with both water-insoluble and water-soluble drugs (5–8). Compared to the solvent evaporation methods, spray-drying has several advantages; the main advantages are that it is a one-step method (5), it has good control of process parameters, and scale-up is easy.

Taking into account all these considerations, the aim of this work was the preparation and characterization of a microparticulate biodegradable drug delivery system loaded with a protein. This system is constituted by microspheres made of PLGA and containing albumin chosen as a model proteic molecule. When spray-drying is used for the preparation of PLGA microspheres containing water-soluble drugs, the more common way is to spray a suspension of the drug in the organic solution of the polymer (6).

In this work, an emulsification/spray-drying method was investigated for the preparation of the protein-loaded PLGA microspheres. A single w/o emulsion containing albumin dissolved in the aqueous dispersed phase and the biodegradable polymer in the organic continuous phase was spray-dried. The rationale was to obtain microspheres containing the protein homogeneously dispersed throughout the polymeric matrix.

EXPERIMENTAL

Materials

The following items were used for the study: bovine serum albumin (BSA), fraction V, essentially fatty acid free (Sigma Chemical Co., St. Louis, MO); poly-D,L-lactide-*co*-glycolide, D,L-lactide : glycolide (PLGA) in a 75 : 25 ratio, Resomer® RG756, M_w approximately 77,000 (by gel permeation chromatography [GPC]) (Boehringer Ingelheim KG, Ingelheim am Rhein) (D). Also used were ethylenoxide/propylenoxide, M_w 8400, Pluronic® F68 (PluF68); polaxamer 407, ethylenoxide 70%/propylenoxide 30%, M_w 12,600, Pluronic® F127 (PluF127) (BASF Co., Chemical Divisions) (D). Sodium oleate (Naol) (Sigma Chemical Co.); dioctyl sodium sulfosuccinate (DSS), M_w 444.6 (Carlo Erba, Milan) (I); BCA Protein Assay Reagent Kit (Pierce, Trimiral S.r.L., Milan) (I); sodium azide (NaN_3), M_w 66.99 (Farmitalia, Milano) (I); and sodium dodecil sulfate (SDS), M_w 288.38 (Janssen Chemicals, Beerse) (B) were used. All other solvents and chemicals were reagent grade.

Methods

Water-in-Oil Emulsification/Spray-Drying Method

The first step consisted of the preparation of the w/o emulsions: Aqueous solutions containing 0.1%

w/v of surfactant (LuF68, LuF127, PluF127, Naol, or DSS) were prepared. BSA (200 mg) was dissolved in 20 ml of these solutions. PLGA was dissolved in 200 ml of methylene chloride (1% w/v) and maintained at a temperature of about 5°C with an ice bath. The aqueous phase was dropwise added into the organic solution under stirring (1500 rpm, Ultra-Turrax, Janke and Kunkel, D), keeping the temperature of the solvents always at about 5°C. The time needed for the complete preparation of the emulsion was about 10–15 min.

The second step consisted of the spraying of the w/o emulsions previously prepared. The spraying process was carried out with a spray-dryer (Mini Spray Dryer Büchi 190, Büchi, CH), cocurrent flow type, equipped with a standard nozzle (0.7 mm inner diameter). The process conditions were always the following, independent of the kind of surfactant used for the preparation of the emulsions: inlet air temperature 59°C–60°C; outlet air temperature 45°C–46°C; feed spray rate about 10 ml/min. The time needed to spray 220 ml of emulsion was about 22 min. The emulsions were always kept under magnetic stirring during the spraying process at a temperature of about 5°C. The solid microparticles obtained from the process were harvested and kept under vacuum for 48 h before use.

Each batch of microspheres was denoted with the abbreviation of the surfactant used for the preparation of the corresponding emulsion.

Emulsion Characterization

The emulsions were examined with an optical microscope at 160× magnification (Zeiss Standard, Zeiss Italia, Arese, Italy), connected to a Reichert camera (Reichert GmbH, A). A picture of the first sample of each emulsion was taken immediately after its preparation. A second picture of a sample of the emulsion was taken 30 min after its preparation (this time approximately corresponds to the time needed to spray 220 ml of emulsion).

Microsphere Characterization

Scanning Electron Microscopy

Scanning electron microscopy (SEM) of the spray-dried microparticles was carried out using a Jeol JXA 840A (Jeol, J) at 8–10 kV acceleration voltage. The microspheres were sputter coated under an argon atmosphere with a thin layer of gold and then photographed.

Particle Size Analysis

Particle size distributions of the spray-dried microspheres were analyzed by the light blockage method with an HIAC/Royco model 3000 (Pacific Scientific, Silver Spring, MD) equipped with an HR60HC sensor. Small amounts of microparticles were suspended in 100 ml of bidistilled filtered water (0.22-μm membrane filters, Millipore S.p.A. Italia, Milan). Then, 10 ml of suspension were withdrawn and analyzed under continuous stirring between 2 and 25 μm. Results are the mean of five withdrawals for each sample tested and are expressed as differential particle size distributions by number.

Drug Content

The determination of the amount of BSA entrapped in the polymeric network of the microparticles was carried out as follows: About 10 mg of microparticles were suspended in 5 ml of a 5% w/v SDS solution in NaOH 0.1 M. The suspension was sonicated for 15 min and then kept at 37°C for 3 days. After this period, the sample was filtered (Millipore 0.45 μm). Of the obtained solution, 100 μl were added to 2 ml of the reactives for protein analyses (BCA). The sample was incubated for 30 min at 60°C and cooled at room temperature. The protein was determined spectrophotometrically (562 nm).

Drug contents are expressed both as encapsulation efficiency and as actual drug content (Table 1). Encapsulation efficiency was calculated from the ratio of actual to theoretical drug content and expressed as a percentage.

In Vitro Dissolution Tests

For the in vitro dissolution tests, 10 mg of microspheres were suspended in 1.5 ml of 0.01 M (pH 7.4) USP phosphate buffer containing 0.01% w/v NaN_3. The suspensions were kept at 37°C. At predetermined time intervals, the microparticles were centrifuged for 20 min, and 1 ml of supernatant was then collected. The supernatant was filtered (Millipore 0.45 μm). Of this solution, 100 μl were added to 2 ml of BCA. The BSA determination was then carried out as previously described for drug content.

Table 1

Characteristics of Microsphere Production

Batch	Yield of Production (%)	Theoretical Drug Content (%)	Actual Drug Content (%)	Encapsulation Efficiency (%)
Pluronic F68	22.58	16.7	12.07	72.4
Pluronic F127	26.67	16.7	14.48	86.8
Sodium oleate	21.41	16.7	13.05	78.3
Dioctyl sulfosuccinate	42.75	16.7	13.17	79.0

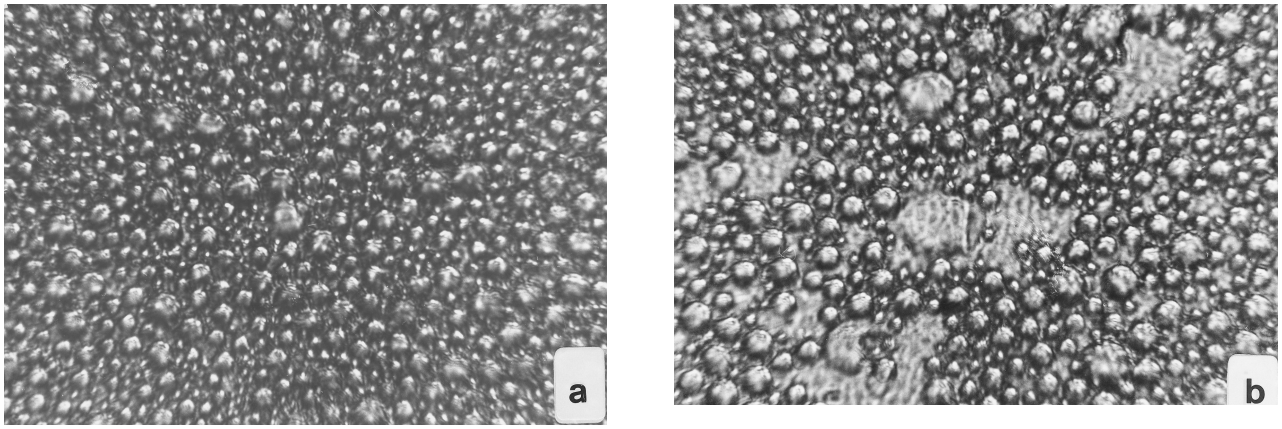

Figure 1. Optical microscopy photographs of the emulsion prepared with PluF127: (a) the emulsion just prepared; (b) the emulsion 30 min after preparation.

RESULTS AND DISCUSSION

Pictures of the emulsion prepared with PluF127, chosen as an example, are reported in Fig. 1. Figure 1a shows the emulsion only just prepared, and Fig. 1b shows the same emulsion after 30 min, which approximately corresponds to the time needed to spray a volume of about 250 ml.

The first picture shows a stable emulsion characterized by the presence of a dispersed phase constituted by droplets of regular shape and uniform size. The characteristics of the system do not appear substantially changed after 30 min, meaning that the emulsion is stable in this time interval. Among all the emulsions prepared, the best results in terms of stability and uniformity of size of the dispersed phase were obtained with PluF127 and DSS. The worst results were obtained with Naol (pictures not shown).

The spraying of the emulsions determines the drying of the solvents and leads to the formation of solid microparticles. The spray-dried microspheres achieved from the more stable emulsions (those prepared with PluF127 and DSS) are also those characterized by the best morphological characteristics in terms of regular spherical shape (Fig. 2a, microspheres obtained using DSS). Microparticles obtained using Naol had an irregular shape and presented some aggregations (Fig. 2b).

Table 1 shows production yields, actual BSA contents, and encapsulation efficiencies of the different batches of spray-dried microparticles. Encapsulation efficiencies were always quite high, between about 73% and 87%, meaning that the incorporation of the protein into the polymeric network of the microparticles was always quite good, independent of the surfactant used.

Production yields were found to be between about 22% and 43%. These low values can be explained both by the relatively low volumes of feed sprayed for the preparation of each batch of microspheres (220 ml for each emulsion) and by the

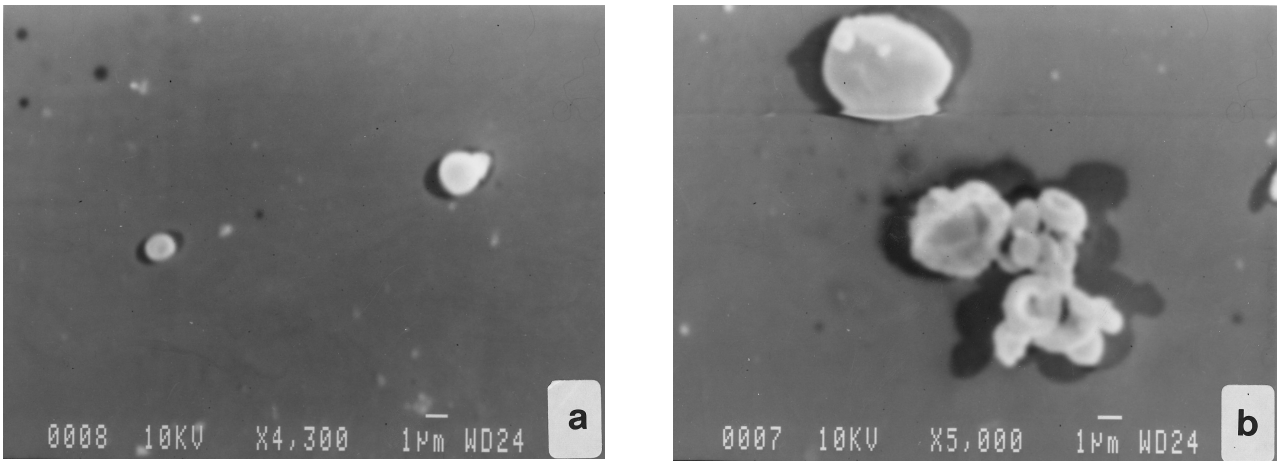

Figure 2. Photomicrographs of PLGA microspheres: (a) microspheres containing PluF127; (b) microspheres containing Naol.

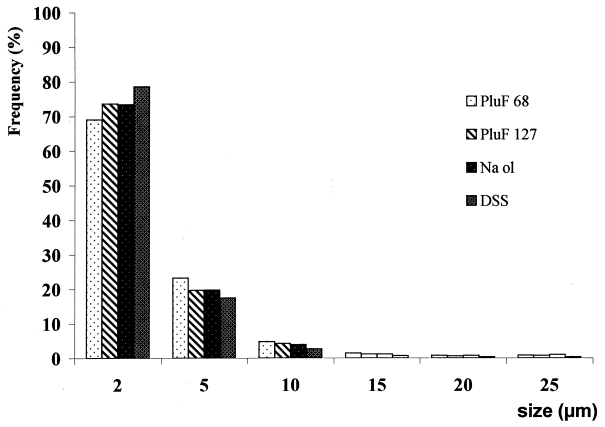

Figure 3. Differential particle size distribution, by light blockage method, of the microspheres.

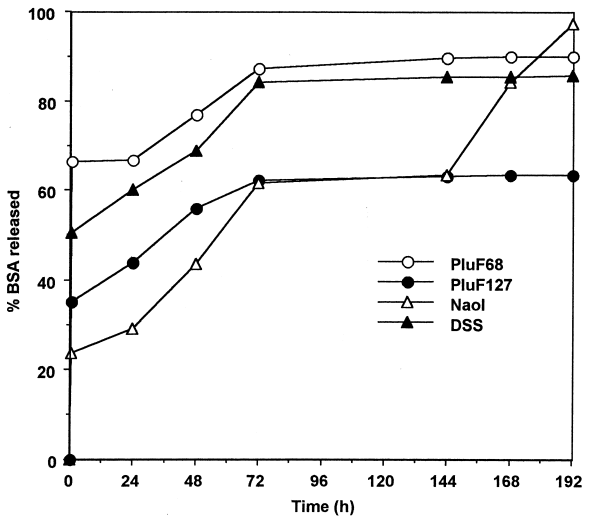

Figure 4. In vitro dissolution profiles of bovine serum albumin released from PLGA microspheres.

structure of the spray-dryer apparatus, which is not equipped with a trap to recover the smaller and lighter particles exhausted by the aspirator.

Results of particle size analyses are reported in Fig. 3 as differential particle size distribution by number. The particle size distribution was not substantially affected by the type of surfactant used as no remarkable differences are evident among the four batches of microparticles prepared. In all cases, the main part of the particles (about 80%) are characterized by a size between 2 and 5 µm. Very few particles having a size larger than 15–20 µm were present.

Figure 4 shows the in vitro release profiles of the different batches of microspheres (USP phosphate buffer, pH 7.4). All batches prepared had bimodal drug release, characterized by an initial burst effect (which ranged in the first 30 min between 20% and about 70%), followed by a slower release rate. The type of surfactant used for the preparation of the emulsion strongly influenced the final result of in vitro release behavior. The following rank order can be found concerning BSA release rate: PluF68 > DSS > PluF127 > Naol. Both the different burst

effects and the slow release rate follow a rank order that is probably due to the different incorporation of the protein into the polymeric network of the microparticles as no substantial differences in particle size were found.

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