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Research paper

Controlling microencapsulation and release of micronized proteins using poly(ethylene glycol) and electrospraying

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ABSTRACT

The fabrication of tailored microparticles for delivery of therapeutics is a challenge relying upon a complex interplay between processing parameters and materials properties. The emerging use of electrospraying allows better tailoring of particle morphologies and sizes than current techniques, critical to reproducible release profiles. While dry encapsulation of proteins is essential for the release of active therapeutics from microparticles, it is currently uncharacterized in electrospraying. To this end, poly(ethylene glycol) (PEG) was assessed as a micronizing and solubilizing agent for dry protein encapsulation and release from electrosprayed particles made from polycaprolactone (PCL). The physical effect of PEG in protein-loaded poly(lactic-co-glycolic acid) (PLGA) particles was also studied, for comparison. The addition of 5–15 wt% PEG 6 kDa or 35 kDa resulted in reduced PCL particle sizes and broadened distributions, which could be improved by tailoring the electrospraying processing parameters, namely by reducing polymer concentration and increasing flow rate. Upon micronization, protein particle size was reduced to the micrometer domain, resulting in homogenous encapsulation in electrosprayed PCL microparticles. Microparticle size distributions were shown to be the most determinant factor for protein release by diffusion and allowed specific control of release patterns.

1. Introduction

Conventional intravenous delivery of therapeutics suffers from excessive dosage being administered and poor bio-availability [1]. In response, the encapsulation of drugs and proteins in carriers has drawn the attention of pharmaceutical research for many decades, yet the systems developed are far from optimal. A resorbable carrier, such as a polymer matrix, has the potential to efficiently protect therapeutic molecules after administration, while providing sustained delivery upon matrix degradation [2]. However, the processes of encapsulation involve organic solvents, shear forces and hydrophobic polymers which may partly denature hydrophilic protein molecules and affect release kinetics [3]. Dry encapsulation of proteins is a strategy to minimize protein denaturation by avoiding water-in-oil interfaces used in traditional techniques, well-acknowledged to denature proteins [4]. While the dry protein is in contact with organic solvent during processing, the solvent provides limited molecular mobility for the protein due to the anhydrous environment. Although the native state of a protein is not favored thermodynamically in such solvents, this combination provides a kinetic trap for the protein, which maintains protein activity [5].

Size reduction of protein aggregates, or micronization, is critical to ensure high, homogeneous, and dispersed protein encapsulation in polymeric carriers upon solvent removal and represents an intricate challenge in the current protein delivery systems. Techniques such as spray-drying and ultrasonic atomization have been used to obtain fine protein particles, but low yields make the techniques unpractical while harsh stresses (mechanical, heat) may lead to

Abbreviations: CLSM, confocal laser scanning microscopy; DCM, dichloromethane; DMF, *N*,*N*-dimethylformamide; EE, encapsulation efficiency; EX, extraction; FITC, fluorescein isothiocyanate; FR, flow rate; GC, group contribution; HRP, horseradish peroxidase; HSP, Hansen Solubility Parameter; L:G, lactide:glycolide; μBCA, micro-bicinchoninic acid; MW, molecular weight; MWD, molecular weight distribution; NIH, National Health Institutes; PBS, phosphate buffer saline; PEG, poly(ethylene glycol); PCL, polycaprolactone; PLA, polylactide; PLGA, poly(lactic-co-glycolic acid); RED, relative energy difference; SA, serum albumin; SDS, sodium dodecyl sulfate; SEM, scanning electron microscope; SOD, superoxide dismutase; TTC, tip-to-collector.

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protein denaturation issues [3]. An easier process was developed by Morita et al., involving co-lyophilization of the protein with poly(ethylene glycol) (PEG) [6]. Lyophilization in the pharmaceutical field has been subjected to ongoing development and is well-known as an approach to overcome the physical and chemical instabilities of protein molecules [7]. When co-lyophilizing PEG and a protein solution, PEG effectively raises the energy barrier for protein molecules to extend their hydrophobic domains to each other, resulting in reduced aggregation with a progressive shrinkage of protein particles [3,8]. Extensive protein conformational changes are also prevented by PEG coating at the surface of proteins, improving both protein stability and delivery capacity [9,10].

Various proteins have been successfully micronized into spherical microparticles with diameters less than 5 μm, including serum albumin (SA), superoxide dismutase (SOD), horseradish peroxidase (HRP) and gelatin, according to different PEG:protein ratios [6,11,12]. Protein aggregate size was found to decrease linearly with the increase in PEG:protein weight ratio and a critical ratio was found, upon which the size decrease was slower. Importantly, the micronization process did not alter the protein and full activity was recovered in the case of SOD [12]. Efficient size reduction, less than 1 μm in diameter, improved the encapsulation efficiency (EE) of active HRP from 24% to 87% [12], and reduced burst release of SA [13] using solid/emulsion-based microencapsulation techniques. It was also shown that proteins and PEG could form stable nano-sized complexes in polar organic solvents by non-covalent interactions, allowing for homogeneous and high EE when dispersed in a PLGA solution and spray-dried [14].

Bioactivity and release profiles can also be efficiently tailored by the use of PEG in formulations since the presence of a hydrophilic additive in a hydrophobic polymer matrix increases diffusion of the encapsulated protein by increasing the degree of pores in the matrix while increasing transport of acidic degradation products away from the matrix [3,15,16]. In a study by Jiang et al., different concentrations of PEG were indeed shown to affect the release of SA from polylactide (PLA) microspheres, with increased release rates for 20% of PEG present in the PLA matrix, compared to 0–10%, but similar profiles were obtained when comparing the inclusions of PEG 10 kDa and 20 kDa [16]. Protein particle size is also critical in directing release profiles with larger protein particles leading to burst release profiles, due to a reduced diffusion of larger protein particles inside a polymer droplet, resulting in an increased protein concentration near the surface of polymeric particles [17]. For micronized proteins, a homogeneous and fine distribution within the particle allows thorough water intrusion, leading to a dense pore network upon release. Such a feature is essential in enabling sustained, reproducible and complete release, although it is currently under-assessed [18].

While several techniques allow solid encapsulation, the emerging technique of electrospraying, in particular, may be highly suited for the efficient encapsulation of therapeutics in polymeric particles [19]. In electrospraying, the protein may directly be dispersed in the polymer solution, which, following subjection to high voltage, results in the extrusion of loaded droplets from a syringe. Droplets undergo solvent evaporation and can be collected, dry, from a conductive substrate. This simple process does not require heat or a sophisticated setup, but involves a complex interplay between processing parameters and polymer solution properties. Nevertheless, our previous reports have shown that a tight control of particle size and morphology can be obtained [20,21], which in combination with dry encapsulation may be suitable in ensuring reproducible release profiles and active proteins being released. However, no reports to date have mentioned or addressed protein micronization prior to electrospraying, which is critical for homogeneous encapsulation of proteins in polymeric particles [19]. Hence, it is hypothesized in this study that PEG may be used as a micronizing agent and a means of tailoring release from electrosprayed particles. A model protein, SA, will be micronized by co-lyophilization with PEG prior to electrospraying and the effect of PEG in the final particle formulation comprising polycaprolactone (PCL) and PLGA 85:15, both FDA-approved polyesters suitable for sustained delivery systems, will be assessed in terms of miscibility of polymers, particle microstructure, protein encapsulation efficiency and protein release.

2. Materials and methods

2.1. Materials

Polycaprolactone (M_n = 84 kDa, PDI 1.53) was obtained from Perstorp Ltd, UK. Poly(lactic-co-glycolic acid) with a lactide:glycolide (L:G) ratio of 85:15 (M_n = 41.3 kDa, PDI 1.6) was purchased from Evonik Industries, USA. Poly(ethylene glycol) with M_n = 6 kDa and M_n = 35 kDa, referred hereafter as PEG 6 k and PEG 35 k, respectively, dichloromethane (DCM), sodium dodecyl sulfate (SDS), serum albumin (SA) and fluorescein isothiocyanate (FITC)-conjugated SA were purchased from Sigma–Aldrich, Australia. Chloroform was purchased from Merck, Germany.

2.2. Particle fabrication

2.2.1. Solid dispersion of dry protein

First, the protein was micronized [6]. Briefly, a series of solutions containing the protein (SA or FITC-SA) mixed with PEG (6 k or 35 k) were freeze-dried. Various polymer solutions made of PCL or PLGA 85:15 were prepared in chloroform or DCM and subsequently added to the protein:PEG lyophilizate under magnetic stirring (see Table S1 in Supplementary Data for details of constituents and ratios). The resultant dispersions were vortexed for 10 s after addition of 1 mL of polymer solution and ultimately probe sonicated for 1 min at 0.5 W (Misonix 3,000, USA) to ensure protein dispersion in the organic solvent.

2.2.2. Electrospraying

Electrospraying was used to produce dried microparticles encapsulating the proteins. Temperature and relative humidity ranged from 23 to 24 °C, and 34% to 49%, respectively. The polymer dispersions were loaded in a 1 mL glass syringe (Hamilton, USA) and extruded through stainless steel nozzles ranging from 26 to 21 G (Terumo, Japan and Becton Dickinson, USA) at constant rates ranging from 0.5 mL/h to 3 mL/h (full details of formulations can be found in Supplementary Data, Table S1) using a syringe pump (World Precision Instruments, USA). A voltage of 10 kV was applied to the needle tip. The tip-to-collector (TTC) distance was either 15 or 25 cm. Collectors consisted of standard aluminum foils (20×20 cm^2) washed with 70% ethanol. After electrospraying, the collectors were placed under vacuum for a further 72 h, to remove any solvent residue. The dry microparticles were then transferred into glass vials and stored at −18 °C until further analysis.

2.3. Physical characterization

2.3.1. Proteins

The dispersion of proteins within electrosprayed particles was assessed using albumin labeled with a florescent dye (FITC). The distribution of FITC-SA within microparticles was visualized using confocal laser scanning microscopy (CLSM). To randomly collect FITC-SA-loaded particles, a microscope glass slide was introduced in the electrospraying apparatus housing and held in contact with the collector, in the center of the spraying zone for 5 min, while electrospraying. The slide was then removed and fluorescence