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Solid state formulations composed by amphiphilic polymers for delivery of proteins: characterization and stability

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ABSTRACT

Nanocomposite powders composed by polymeric micelles as vehicles for delivery proteins were developed in this work, using insulin as model protein. Results showed that size and polydispersity of micelles were dependent on the amphiphilic polymer used, being all lower than 300 nm, while all the formulations displayed spherical shape and surface charge close to neutrality. Percentages of association efficiency and loading capacity up to 94.15 ± 3.92 and 8.56 ± 0.36, respectively, were obtained. X-ray photoelectron spectroscopy (XPS) measurements confirmed that insulin was partially present at the hydrophilic shell of the micelles. Lyophilization did not significantly change the physical characteristics of micelles, further providing easily dispersion when in contact to aqueous medium. The native-like conformation of insulin was maintained at high percentages (around 80%) after lyophilization as indicated by Fourier transform infrared spectroscopy (FTIR) and far-UV circular dichroism (CD). Moreover, Raman spectroscopy did not evidenced significant interactions among the formulation components. The formulations shown to be physically stable upon storage up to 6 months both at room-temperature (20 °C) and fridge (4 °C), with only a slight loss (maximum of 15%) of the secondary structure of the protein. Among the polymers tested, Pluronic® F127 produced the carrier formulations more promising for delivery of proteins.

1. Introduction

In the last decades, the use of polymers in the development of drug delivery systems has gained a new breath as consequence of the progresses seen in the fields of polymer engineering and nanotechnology applied to health. Among them, amphiphilic polymers have emerged as platforms for advanced delivery of a variety of drugs (Andrade et al., 2011a). The most commonly used are poloxamers, triblock copolymers of polyoxyethylene and polyoxypropylene, commercially known as Pluronic® (Kabanov et al., 2002). However, poly(D,L-lactide-co-glycolide)-b-poly(ethylene-glycol) (PLGA-PEG), poly(ε-caprolactone)-b-poly(ethylene-glycol) (PCL-PEG) as well as their derivatives (Song et al., 2011; Moretton et al., 2013) are also commonly used. Nanotechnology-based delivery systems have been explored to solve the drawbacks of conventional formulations such as instability and degradation, reduced permeation through biomembranes and bioavailability (Andrade et al., 2011b). Polymeric micelles are spherical shape nano-sized structures composed by amphiphilic polymers or

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polymers conjugated with lipids that are suitable as drug delivery systems. The inner core of micelles presents the capacity to encapsulate hydrophobic drugs, while the shell can associate the hydrophilic ones (Kedar et al., 2010). Due to its small size, micelles generally escape from the reticulo-endothelial system, presenting higher bloodstream circulation time (Andrade et al., 2011a). Also, some studies suggest the capacity of polymeric micelles to inhibit the drug efflux mechanisms and consequently multidrug resistance (Andrade et al., 2013). In addition, similar to liposomes, the surface of polymeric micelles can be easily tailored with specific ligands for targeted delivery (Chen et al., 2008). Nevertheless, micelles present the advantage of being more stable than liposomes (Andrade et al., 2011a). The versatility of micelles explains why they have been proposed as vehicles for solubilization and delivery of a variety of drugs like doxorubicin (Chen et al., 2012), paclitaxel (Kato et al., 2011; Lee et al., 2008), rifampicin (Moretton et al., 2013), calcitonin (Baginski et al., 2012), cyclosporine A (Di Tommaso et al., 2012) among others, being some formulations in clinical trials (Kato et al., 2011; Lee et al., 2008). Due to the development noted in the biotechnology field, biopharmaceuticals have emerged as an alternative to conventional drugs in the treatment of many diseases. Since the commercialization of insulin obtained by biotechnology processes in 1982, biopharmaceuticals have gained an increased share in the global pharmaceutical market (EvaluatePharma, 2012). Despite their well-known therapeutic efficacy, the major drawback of biopharmaceutical drugs is the difficulty of administration *via* non-invasive routes in their active conformation. The objective of the present work was the development of a system for administration of biopharmaceuticals, using insulin as a model protein. The system comprised polymeric micelles composed by amphiphilic polymers, namely polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer (Soluplus® (SOL), Fig. S1 of Supplemental material) or polyethylene glycol (PEG)-polypropylene oxide (PPO)-polyethylene glycol (PEG) block copolymer (Pluronic® F68 (F68), Pluronic® F108 (F108) and Pluronic® F127 (F127) (Fig. S1 and Table S1 of Supplemental material)) in which insulin was encapsulated. The development of stimuli-sensitive formulations with the addition of phenylboronic acid (PBA) to the system was also hypothesized. Boronic acid derivatives have been proposed as excipients to control the release of insulin from formulations as response to glucose concentration (Cambre and Sumerlin, 2011). Neutral boronic moieties convert to anionic boronate esters upon reaction with the diol group of sugars, increasing the hydrophilicity of the system. Many hydrogels containing boronic acid derivatives have been shown to swallow and release insulin as response to the increase in the hydrophilicity (Cambre and Sumerlin, 2011). Here are reported the results regarding the development and production of micelles and powder formulations, their physical and chemical characterization as well as the assessment of insulin structure after production and storage. To our knowledge, no other report regarding Soluplus® as vehicle to delivery biopharmaceutical drugs, namely proteins, has been ever reported.

2. Materials and methods

2.1. Materials

SOL, F68, F108 and F127 were kindly provided by BASF (Ludwigshafen, Germany). Lyophilized human insulin, PBA and phosphate buffer saline pH 7.4 (PBS) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The other reagents used were methanol and ethanol absolute from analytical grade; acetonitrile and trifluoroacetic acid (TFA) from HPLC grade (Merck, Germany) and Type 1 ultrapure water (18.2 MΩ cm at 25 °C, Milli-Q®, Billerica, MA, USA).

2.2. Production of micelles

Micelles were prepared using the thin-film hydration technique. Briefly, each polymer was individually weight and dissolved in a mixture of methanol:ethanol (1:1). Then, the solvent was removed under vacuum and the film was left to dry overnight at room-temperature to eliminate any remained solvent. The film was then hydrated with PBS at 37 °C in order to obtain a 1% (w/v) polymer solution and vortexed for 5 min. The obtained dispersion was filtered through a 0.22 μm syringe filter to remove possible dust and aggregates.

PBA containing micelles were prepared by dissolving PBA with the polymers in the solvents prior to the production of the film at a ratio of 10:1 (polymer:PBA). Insulin formulations were prepared by adding different amounts of insulin in the form of solution in PBS during the film hydration, to obtain polymer:insulin ratios ranging from 10:0.1 to 10:1 (w/w). The other steps were the same as for plain formulations. After preparation, the pH of all formulations was measured, ranging between 6.1 and 7.1.

2.3. Determination of size, zeta potential and association efficiency of formulations

Micelles were characterized regarding size, surface charge and association efficiency. Full description of the assays is detailed in Supporting information, Section S1.

2.4. Lyophilization

After production the formulations were lyophilized in an AdVantage 2.0 BenchTop Freeze Dryer (SP Scientific, Warminster, PA, USA) in order to increase their stability and to study the rehydration of micelles in water. Formulations were also lyophilized to obtain powders for characterization. The cycle used was the follow: the samples were frozen at −30 °C and the temperature maintained for 60 min, the primary drying was set at 20 °C for 480 min at 150 mTorr and the secondary drying for another 480 min at 30 °C and 100 mTorr.

2.5. Morphological characterization of micelles

Different microscopic techniques, namely atomic force microscopy (AFM), field emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM), were used to characterize the morphology of the micelles. Full description of the techniques is detailed in Supporting information, Section S1.

2.6. Thermal analysis

The thermal behavior of the pure compounds, physical mixtures (1:1) and lyophilized formulations was assessed by DSC. Thermograms were obtained using a Shimadzu DSC-60 system (Shimadzu, Kyoto, Japan). 5 mg of each powder sample in an aluminum crimp was exposed to a controlled thermal treatment, specifically heated from 30 to 300 °C at a rate of 10 °C/min under constant purging of nitrogen at 40 mL/min, and the heat flow measured.

2.7. XRD experiments

Crystallization properties of powder samples were analyzed by XRD. Spectra were acquired using X'Pert PRO MPD θ/θ powder diffractometer of 240 mm of radius (PANalytical B.V., Almelo,