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Insulin-loaded poly-L-lactide porous microspheres prepared in supercritical CO_2 for pulmonary drug delivery

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ABSTRACT

The insulin-loaded poly-L-lactide porous microspheres (INS-PLLA PMs) were successfully developed in an emulsion-combined precipitation of compressed CO_2 antisolvent (PCA) using ammonium bicarbonate (AB) as a porogen. The resulting INS-PLLA PMs exhibited a rough and porous structure with a geometric mean diameter (D_g) of 15.62 μm, an aerodynamic diameter (D_a) of 4.31 μm, a fine particle fraction (FPF) of 65.57% and good aerosolization characteristics. The physicochemical characterization reveals that no chemical changes occurred on INS-PLLA PMs, while minor structural changes existed in insulin. The result of circular dichroism (CD) spectroscopy demonstrates a slight change happened in the secondary structure of insulin, however, the bioactivity verification test shows that the hypoglycemic activity of insulin from INS-PLLA PMs was well maintained, which shows no significant difference from the raw insulin. The fluorescent image of INS-PLLA PMs demonstrates that the insulin was homogeneously distributed in the matrix, and INS-PLLA PMs displayed a sustained-release effect. Furthermore, INS-PLLA PMs with almost no organic residue could promote the safety and suitability for pulmonary delivery of protein drugs. This study indicates that emulsion-combined PCA process is an effective and benign technology to produce INS-PLLA PMs, which have potential in the application of pulmonary drug delivery for treatment of diabetes.

1. Introduction

Pulmonary drug delivery by dry powder inhalation is a considerable alternative for delivery of therapeutic drugs such as peptides, proteins, gene and vaccine [1–4], which should otherwise be injected. The pulmonary drug delivery possesses non-invasiveness and remarkable bioavailability when compared with other administration routes. These are attributed to the physiological features of the lung, which displays a more than 100 m^2 of surface area, high vascularization, thin blood-alveolar barrier, inferior enzyme activities and the absence of hepatic first-pass effect [5–7]. Since the Exubera®, the insulin-based dry powder approved by FDA via pulmonary delivery for diabetes treatment, was withdrawn from the market, the other insulin preparations via pulmonary route have been extensively studied [8–11].

The large porous particle for pulmonary drug delivery was first proposed by Edwards in 1997 [12]. The large porous particle with a low value of aerodynamic diameter (D_a) as well as a reasonably high value of geometric mean diameter (D_g) could not only achieve an effective lung deposition [13,14] and prolong the residence time in lung, but also improve the stability and sustained-release effect of the macromolecular drugs [15–17]. The representative strategies for preparation of large porous particles can be summarized as spray-drying [18–20] and double emulsion-solvent evaporation [21–23]. The spray-drying method is not suitable for temperature-sensitive drugs and the resulting porosity is relatively low [24]. The double emulsion-solvent evaporation method needs appropriate pore-forming agents and further treatments must be carried out to reduce the organic solvent residue of the product. Using the cyclodextrin as a porogen, Ungaro et al. [15] produced the inhalable insulin-loaded PLGA large porous particles by a double emulsion method; the resulting particles displayed a fine aerodynamic property and a considerable hypoglycemic activity in the diabetic rats.

Notably, supercritical CO_2 has been exhibiting great advantages in the field of drug carrier preparations due to its low organic solvent residue, moderate operating condition and environmental friendliness [25–28]. Falco et al. [29] used the continuous supercritical emulsions extraction process to prepare the insulin-loaded PLGA microspheres with a low residual solvent; and the process

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was appropriate for the preparation of the thermolabile compounds. For the large porous particle fabrication, supercritical CO_2 can be used as drying agent for polymer gel [30,31], gas foaming agent in polymer microspheres [32] or porogen in polymerization reaction system [33,34]. Nevertheless, these methods generally suffered a low porosity and difficulty in loading drugs into carriers by one-step process, or required a high temperature for some foaming processes, which are not suitable for producing proteins-loaded large porous particles for pulmonary drug delivery. Dhanda et al. [33] used a solvent evaporation technique to produce the celecoxib loaded PLGA microspheres, followed by a supercritical CO_2 based pressure-quench technology to develop the large porous structures for pulmonary drug delivery; the resulting porous microspheres displayed a low porosity and slight residual solvents.

Herein, we attempted to employ an emulsion-combined precipitation of compressed CO_2 antisolvent (PCA) process with ammonium bicarbonate (AB) as a porogen for the preparation of protein-loaded porous microspheres for pulmonary drug delivery [35,36], which would avoid the drawbacks of organic solvent residue and low porosity caused by the conventional methods. In this study, we chose insulin as a protein drug model to prepare insulin-loaded poly-L-lactide porous microspheres (INS-PLLA PMs) for treatment of diabetes via pulmonary delivery. The relating properties including morphology, particle size, aerodynamic properties, physicochemical properties, hypoglycemic activity, drug loads, encapsulation efficiencies and drug release profiles were characterized.

2. Materials and methods

2.1. Materials

PLLA (MW 50,000, 1.5 dL/g) was purchased from the Jinan Daigang Co., Ltd. (Jinan, China). Pluronic F-127 (PF-127) and AB were purchased from Sigma–Aldrich (USA). Insulin from bovine pancreas (>27 IU/mg, 98% purity) was purchased from Dalian Meilun Biological Technology Co., Ltd. (Dalian, China). Dichloromethane (DCM, 99.8% purity) was purchased from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). CO_2 of 99.9% purity was supplied by the Rihong Air Products Co., Ltd. (Xiamen, China). Fluorescein isothiocyanate (FITC) was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Bicinchoninic Acid (BCA) Kit was purchased from Applygen Technologies Inc. (Beijing, China). All other compounds were of analytical purity.

Adult male Kunming (KM) mice weighed of 30–35 g were purchased from the Slac Laboratory Animal Co., Ltd. (Shanghai, China). Food and water were available ad libitum. Mice were kept in a facility under a 12 h light/dark cycle.

2.2. Methods

2.2.1. Preparation of INS-PLLA PMs by emulsion-combined PCA process

The INS-PLLA PMs were prepared by an emulsion-combined PCA process as previously reported [35,36]. Briefly, 306.7 mg of PLLA and 153.3 mg of PF-127 were dissolved in 20 mL DCM as the oil phase. The water phase consisted of insulin and 2 mL of AB aqueous solution at a concentration of 250 mg/mL. The water phase was added into the oil phase, followed by an ultrasonic emulsification at 200 W for 1 min. The finally-prepared emulsion was then subjected to the PCA process. As shown in Fig. 1, the apparatus consists of a CO_2 supply system, a solution delivery system and an autoclave with a volume of 500 mL. In a PCA process, the CO_2 was cooled down to around 0 °C by using a cooler, followed by delivery using a high pressure meter pump. Then a heat exchanger was used to preheat the liquefied CO_2. When the operating parameters reached at 8 MPa and 30 °C, we adjusted the frequency of CO_2 pump to maintain a steady flow of CO_2 (1000 L/h). The emulsion was fed into the autoclave through a nozzle at a flow rate of 4.0 mL/min. When the emulsion was completely injected into the autoclave, a washing process was performed by the compressed CO_2 for about 30 min under the same conditions described in above. This was to remove the residual organic solvent. Next, the autoclave was slowly depressurized and the microspheres containing AB could be obtained. The resulting microspheres were dried using vacuum drying at 35 °C for 2 h to decompose AB. Finally, INS-PLLA PMs were harvested for further characterizations.

2.2.2. Morphology and particle size characterizations

Surface morphological examinations were carried out by a field-emission scanning electron microscope (FE-SEM S-4800, Hitachi, Japan). The samples were absorbed onto the conducting resin and then sprayed with gold under vacuum conditions.

The D_g and particle size distribution were investigated by Laser particle size analyzer (LS13-320, Beckman Coulter, USA). For the aerodynamic property, a lower mass median aerodynamic diameter (MMDA) was adopted as D_a, which was measured by an eight-stage Andersen Mark II cascade impactor (ACI 20-810, Thermo Scientific, USA) to speculate the practical deposition into the lung [17,37]. The capsule containing 10 mg of the INS-PLLA PMs was placed in a dry powder inhaler device and a hole was made so that the powder could be released. Then the samples were absorbed into the ACI at a flow rate of 28.3 L/min. Finally, the weight of powder at each stage was recorded for the calculation of the D_a and the fine particle fraction (FPF).

2.2.3. Aerosolization properties analysis

To study the aerosolization of INS-PLLA PMs, photos were captured at 0.04 s intervals after actuation using a digital camera (D3100, Nikon, Japan) [22]. The aerosolization efficiencies by an insufflator device (DP-4) and an air pump (AP-1) (Penn-Century, Inc., Philadelphia, PA) were researched. The air volume was set at 2.0 mL for a single actuation. Data are presented as means ± SDs of three independent.

2.2.4. Fourier transform infrared (FTIR) analysis

For FTIR analysis, samples were separately mixed with KBr and measured by FTIR analyzer (8400S, Shimadzu, Japan) in the transmission mode, with the wavenumber ranging from 4000 cm^{-1} to 400 cm^{-1}.

2.2.5. Gas chromatography (GC) analysis of organic solvent residue

GC (6890N, Agilent Technologies Inc., USA) was used to detect the residual organic solvent in the INS-PLLA PMs. Approximately 500 mg of sample was accurately weighed, and the analysis was performed by the static head-space method.

2.2.6. Circular dichroism (CD) spectroscopy analysis

For CD spectroscopy analysis, a 0.1 mm path cell for far-UV CD was used. The parameters were as follows: a scanning wavenumber of 190–250 nm, a scanning speed of 100 nm/min, a flow rate of N_2 at 5–10 L/min and sensitivity of 5 mdeg/cm. CD spectrum of the protein analysis was removed from the reference set and the secondary structure fractions were determined using computer programs CDSSTR in CDpro software [38]. Each sample was carried out in independent triplicate.

2.2.7. Bioactivity verification test

For the bioactivity verification test, KM mice were randomly divided into three groups. The mice were fasting for 12 h and