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Research paper

Spray freeze drying for dry powder inhalation of nanoparticles

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ABSTRACT

Formulating nanoparticles for delivery to the deep lung is complex and many techniques fail in terms of nanoparticle stability. Spray freeze drying (SFD) is suggested here for the production of inhalable nanocomposite microcarriers (NCM). Different nanostructures were prepared and characterized including polymeric and lipid nanoparticles. Nanoparticle suspensions were co-sprayed with a suitable cryoprotectant into a cooled, stainless steel spray tower, followed by freeze drying to form a dry powder while equivalent compositions were spray dried (SD) as controls. SFD-NCM possess larger specific surface areas (67–77 m^2/g) and lower densities (0.02 g/cm^3) than their corresponding SD-NCM. With the exception of NCM of lipid based nanocarriers, SFD produced NCM with a mass median aerodynamic diameter (MMAD) of 3.0 ± 0.5 μm and fine particle fraction (FPF ⩽ 5.2 μm) of 45 ± 1.6% with aerodynamic performances similar to SD-NCM. However, SFD was superior to SD in terms of maintaining the particle size of all the investigated polymeric and lipid nanocarriers following reconstitution (S_f/S_i ratio for SFD ≈ 1 versus >1.5 for SD). The SFD into cooled air proved to be an efficient technique to prepare NCM for pulmonary delivery while maintaining the stability of the nanoparticles.

1. Introduction

Over the last decades, colloidal drug delivery systems and especially nanoparticles have received increasing attention. Nanosystems with different compositions and biological properties have been extensively investigated for drug, protein and gene delivery applications. Pulmonary delivery has become a popular method to deliver therapeutic or diagnostic compounds. This is due to the large alveolar surface area, the low thickness of the epithelial barrier and the extensive vascularization in the alveolar region [1,2]. The use of nanoparticles as therapeutic carriers for pulmonary delivery has gained significant interest because of their ability to enter the intracellular compartments and their bioavailability enhancement potential attributed to the unique ability of nanoparticles to evade the alveolar macrophages and mucociliary clearance mechanisms, resulting in prolonged drug residence time [3].

Nanoparticle delivery to the lungs suffers from two major drawbacks: firstly, nanoparticles, with the exception of particles <50 nm in size, are exhaled from the lungs [3]. Secondly, they show formulation instability due to their high surface energy, leading to aggregation and/or particle–particle interactions [4]. To overcome these problems, nanoparticles are often applied to the lungs in the form of suspensions. However, in this case, the size of the generated droplets will vary with the nebulizer technique and the applied stress during nebulization can affect the formulation stability [5]. The frequent instability problems of nanosuspensions, such as aggregation and/or drug leakage, can be overcome by applying nanoparticles as dry powder. Therefore, the production of nanocomposite microcarriers (NCM) has been suggested as a possible formulation strategy [6–8]. The formulation into a microsized carrier improves the stability and aerodynamic properties of the entrapped nanoparticles [9,10]. In this case, the size of the microcarrier determines the deposition in the lungs and is independent on the application device.

Abbreviations: SFD, spray freeze drying; SD, spray drying; NCM, nanocomposite microcarriers; NP, nanoparticles; PLGA, poly (DL-lactide-co-glycolide); EDRL, poly(meth)acrylate (Eudragit® RL PO); EC, ethyl cellulose; PVAL, polyvinyl alcohol; MCT, medium chain triglyceride; PVP, polyvinylpyrollidone (kollidon 12 PF); LNC, lipid nanocapsules; SLN, solid lipid nanoparticles; PDI, polydispersity index; SEM, scanning electron microscope; d_{50}, volume median particle size; ρ_{bulk}, bulk denisity; ρ_{tapped}, tapped density; CI, Carr's compressibility index; NGI, next generation impactor; DPI, dry powder inhaler; MOC, micro-orifice contactor; RD, recovered dose; EF, emitted fraction; FPF, fine particle fraction; MMAD, mass median aerodynamic diameter; GSD, geometric standard deviation; S_f, particle size after reconstitution; S_i, particle size before reconstitution.

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Spray drying (SD) is the commonly used technique to produce NCM within the aerodynamic diameter range suitable for pulmonary deposition [7–14]. One prominent concept was introduced by Tsapis et al. [15] who prepared an extremely thin-walled macroscale structures by spray drying solutions of non-polymeric and polymeric nanoparticles. The solutes and nanoparticles accumulate at the evaporating front of the droplet and form a shell that dries to become the hollow microparticle. However, SD has some limitations regarding its use for heat sensitive materials and it requires precise adjustment of the inlet and outlet temperatures of the used hot gas. Alternatively, we propose here SFD for the production of inhalable NCM. In SFD, nanoparticle dispersions are atomized into a stainless steel spray tower encased by a cooling jacket of liquid nitrogen, so that the particles are freezed during the time of flight in the cold air, avoiding any contact with liquid nitrogen [16]. Additionally, a two-fluid nozzle was used to produce droplets of a size range suitable for pulmonary deposition. The two major prerequisites for these NCM are a significant lung deposition and a full reconstitution of nanoparticles when coming in contact with aqueous liquid, to maintain their beneficial therapeutic characteristics.

In this study, the feasibility of using SFD for preparing inhalable NCM of polymeric and lipid nanoparticles was investigated. Different polymeric and lipid nanostructures were prepared in order to test the effect of the nanocarrier type on the process of microcarrier formulation. Poly (DL-lactide-co-glycolide), Eudragit® RL and ethyl cellulose were investigated as polymer candidates for polymeric nanoparticles, while lipid nanocapsules and solid lipid nanoparticles were tested as lipid based nanocarriers. Afterward, the prepared polymeric and lipid nanostructures were co-spray freeze dried with maltodextrin and trehalose, respectively. Nevertheless, a comparative study was carried out with samples similarly prepared using SD technique. This comparison took into consideration: the production feasibility; the suitability of the prepared micro-carriers for pulmonary deposition; reconstitution ability of the NCM and nanoparticles size after reconstitution.

2. Experimental

2.1. Materials

Poly (DL-lactide-co-glycolide) (Resomer® RG 502 H; PLGA) was obtained from Boehringer Ingelheim, Germany. Poly(meth)acrylate: Eudragit® RL PO (EDRL) was a kind sample from Evonik Röhm GmbH, Darmstadt, Germany. Ethyl cellulose (Ethocel standard 4 premium, EC) was kind gift from Colorcon, UK. Miglyol® 812 (medium chain triglyceride, MCT) was from Fagron GmbH, Barsbüttel, Germany. Soybean lecithin and polysorbate 80 (Tween® 80) were purchased from Caelo, Germany. Witepsol® H15 was from Sasol GmbH, Witten, Germany. Polyvinyl alcohol (PVAL) 98–99% hydrolyzed and cholic acid sodium salt hydrate were purchased from Sigma–Aldrich Chemie GmbH, Steinheim, Germany. Maltodextrin (Roquette LAB 2509, dextrose equivalent of DE = 19) was a gift from Roquette Freres, Lestrem Cedex, France. Polyvinylpyrollidone (kollidon 12 PF, *K*-value range = 10.2–13.8, PVP), Cremophor® A25 and Kolliphor® HS15 were kind samples from BASF, Ludwigshafen, Germany. Trehalose (Ph. Eur.) was purchased from VWR International, Amsterdam, Netherlands. All other chemicals were of analytical grade or equivalent purity.

2.2. Preparation of nanoparticles

Polymeric nanoparticles were prepared by the o/w emulsion solvent evaporation technique [17,18], using poly DL-lactide-co-glycolide (PLGA), ethyl cellulose (EC) or Eudragit RL (EDRL) as a polymer. 0.5 g. of each of the investigated polymers was dissolved in 25 ml of either dichloromethane (for EDRL) or ethyl acetate (for PLGA and EC), forming the organic phase. This organic solution was then poured into 50 ml of the aqueous surfactant solution (0.1% sodium cholate for PLGA, 0.1% Tween 80 for EDRL, 1% PVAL for EC). The coarse emulsion formed was then further homogenized at 50 W for 5 min using ultrasonic cell disruptor (Banoelin sonopuls, Berlin, Germany). The solvent evaporation step was performed using a Büchi Rotavapor RE120 (Büchi, Flawil, Switzerland) for 20 min, reducing the pressure stepwise down to 30 mbar with a diaphragm pump.

Lipid nanocapsules (LNC) were prepared according to a solvent-free phase inversion method that allows the preparation of very small nanocapsules by thermal manipulation of oil/water system [19,20]. Briefly, 1 g. of the oil phase (triglyceride phase, MCT) was mixed with 1 g Kolliphor® HS15 and 3 g distilled water. Sodium chloride (100 mg) and soybean lecithin (100 mg) were also added. The mixture was heated under magnetic stirring up to 85 °C (until a distinct drop of conductivity occurs) to ensure that the phase inversion temperature was passed and a w/o emulsion was formed. Afterward, the emulsion was allowed to cool down to 55 °C on another magnetic stirrer. During cooling, another complete phase inversion to an o/w emulsion occurs. This cycle was repeated twice before adding 5 ml of distilled water at 4 °C. The LNC suspension was then stirred for 10 min before further analysis.

Solid lipid nanoparticles (SLN) were prepared by melting 10 g of the solid lipid Witepsol at 70 °C. The aqueous phase consisting of 90 ml water, containing 1 g sodium cholate and 2.5 g Cremophor® A25, was also heated to the same temperature and then added to the lipid melt followed by homogenization with ultraturrax at 10,000 rpm for 10 min. The hot emulsion was then sonicated using ultrasonic cell disruptor (Banoelin sonopuls, Berlin, Germany) for 20 min at 70 °C and left overnight before further investigations [17,21].

2.3. Determination of the particle size

The prepared nanoparticles were analyzed for their particle size and size distribution in terms of the average volume diameters and polydispersity index (PDI) by photon correlation spectroscopy using particle size analyzer (Brookhaven Instruments Corporation, Holtsville, NY, USA) at fixed angle of 90° at 25 °C. The nanoparticle suspension was diluted with distilled water before particle size analysis. All samples were analyzed in triplicates at 25 °C and the error was calculated as standard deviation (SD).

2.4. Spray drying

Nanoparticle dispersions (1% *w*/*v*) were mixed with 5% *w*/*v* of either maltodextrin (for polymeric nanoparticles) or trehalose (for lipid nanocarriers) and 5% *w*/*v* PVP as stabilizers. The dispersions were then spray dried using a Büchi B-191 mini Spray Dryer (Büchi, Flawil, Switzerland) equipped with a two-fluid nozzle (0.7 mm). Spray drying was undertaken with the following settings: feed rate 3% (1 ml/min), inlet temperature 110 °C, air flow rate 750 NL/h and aspiration 85%. These settings resulted in an outlet temperature of 80 °C. Florescent microcarriers were prepared by incorporating 0.05% *w*/*v* sodium fluorescein in the aqueous suspension before spraying. The obtained powder was stored in vacuum desiccator over silica gel until used.

2.5. Spray freeze drying

SFD was carried out according to the method described by Eggerstedt et al. [16] with some modifications. Briefly, the process consisted of three steps: droplet formation, freezing, and freeze drying. For droplet formation, a two-fluid nozzle (0.7 mm) was