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Optimization of the aerosolization properties of an inhalation dry powder based on selection of excipients

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ABSTRACT

The aim of this study was to investigate the influence of formulation excipients on physical characteristics of inhalation dry powders prepared by spray-drying. The excipients used were a series of amino acids (glycine, alanine, leucine, isoleucine), trehalose and dipalmitoylphosphatidylcholine (DPPC). The particle diameter and the powder density were assessed by laser diffraction and tap density measurements, respectively. The aerosol behaviour of the powders was studied in a Multi-Stage Liquid Impinger. The nature and the relative proportion of the excipients affected the aerosol performance of the powders, mainly by altering powder tap density and degree of particle aggregation. The alanine/trehalose/DPPC (30/10/60 w/w/w) formulation showed optimal aerodynamic behaviour with a mass median aerodynamic diameter of 4.7 μm, an emitted dose of 94% and a fine particle fraction of 54% at an airflow rate of 100 L/min using a Spinhaler inhaler device. The powder had a tap density of 0.10 g/cm^3. The particles were spherical with a granular surface and had a 4 μm volume median diameter. In conclusion, optimization of the aerosolization properties of inhalation dry powders could be achieved by appropriately selecting the composition of the particles.

1. Introduction

Inhalation of small molecule drugs and biopharmaceuticals is an efficient and convenient local drug delivery method [1]. It allows the targeted therapy of the diseased airways with high drug concentrations at the site of action and low systemic drug exposure (and thereby reduced systemic side effects). Inhalation aerosols have also been developed for systemic drug administration. Yet, chronic insulin delivery to the lung has encountered several setbacks, including the increased incidence of lung cancer [2], and pharmaceutical companies have stopped marketing and development of the product. Systemic drug delivery by inhalation might still be envisioned but it might be more suitable for drugs used to treat acute conditions such as migraine (e.g., ergotamine [3]) and which do not present growth factor properties as insulin does.

Particles to be inhaled need to be within the 1- to 5-μm aerodynamic diameter range in order to reach the airways [1]. The devices capable to generate these particles include nebulizers, pressurized metered-dose inhalers and dry powder inhalers (DPIs). DPIs present advantages over the other systems [4]. The fine particle fractions (FPFs), that is, the fraction of particles and particle aggregates with an aerodynamic size smaller than 5 μm, are high using simple and cheap inhaler devices. Dry powder inhalers are robust, portable, propellant-free and breath-actuated. The solid state provides a more stable environment for the drug than the liquid state. Dry powders do not need to be sterile.

Micronization is the conventional method for the preparation of inhalation dry powders [5]. It uses jet milling to reduce the size of crystalline material into fine aerosol particles. Yet, little control is obtained over particle size, shape and surface morphology, and the powders produced are highly cohesive and present poor aerosolization properties. Since device technology could not strongly improve aerosolization of micronized powders, the need for improved dry powder formulation became evident. At the start of the 1990s, spray-drying was developed as an alternative method to micronization, especially for the development of protein inhalation powders [5–6]. Spray-drying is a one-step process that converts a liquid feed to a dry powder form. The technique provides control over particle size, shape and surface properties, and aerosolization can therefore be made easier. Excipients can be included in the solution feed in order to obtain chemically stable and dispersible dry powder formulations [7].

Getting a know-how in formulation excipients is essential for the optimization of spray-dried powders [7]. We have previously studied albumin, lactose, and dipalmitoylphosphatidylcholine (DPPC) as excipients for preparing inhalation dry powders [8–9]. The combination of albumin, lactose and DPPC in the proportion 30/10/60 by weight yielded a free-flowing dry powder with

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a primary geometric particle diameter of 4.7 μm, a tap density of 0.05 g/cm^3 and an emitted dose of 86% and FPF of 52% in a Multi-Stage Liquid Impinger (MSLI) operated at 60 L/min [10]. Although the dry powder presented good aerosolization properties, drawbacks are linked to lactose and albumin. Lactose is a reducing sugar that has the potential to react with functional groups of small molecule drugs or lysine residues of protein drugs [11]. Albumin, being a protein, shows a limited shelf stability and can induce the production of auto-antibodies following repeated administration [6]. Moreover, the animal source of both lactose (bovine) and albumin (human) entails risks of toxicity and infection due to impurities [11].

The aim of this study was to produce inhalation dry powders by spray-drying using several alternative excipients. Amino acids with increasing hydrophobicity were selected: glycine, alanine, leucine and isoleucine. Amino acids can enhance the aerosol behaviour of spray-dried powders by reducing moisture sorption and surface tension [12–14]. Amino acids can also protect proteins against thermal stresses and denaturation [15]. Trehalose was used as alternative to lactose because it does not exhibit a reducing character and it additionally stabilizes proteins during drying [16]. DPPC was kept as principal excipient because it improves the aerodynamic characteristics of aerosols and has the potential to stabilize protein drugs during spray-drying [17–18]. In addition, DPPC is biocompatible since it is the major phospholipid of lung surfactant [19]. We studied the influence of the different excipients and of their proportions on the physical characteristics of the powders and identified the formulation with optimal aerodynamic properties. The particle diameter was measured by laser diffraction and the powder density by tap density measurements. The aerosol behaviour was assessed in a MSLI using a Spinhaler inhaler device.

2. Materials and methods

2.1. Chemicals

D-trehalose dihydrate, sulforhodamine 101 and human serum albumin (fraction V, 96–99% albumin) were obtained from Sigma (Sigma–Aldrich, Bornem, Belgium). Dipalmitoyl phosphatidylcholine (DPPC) was purchased from Lipoid (Lipoid GMBH, Ludwigshafen, Germany). L-alanine, glycine, L-isoleucine, L-leucine and ethanol absolute 99.8+% were supplied by Acros Organics (Geel, Belgium). $NaH_2PO4{\cdot}1H_2O$, $Na_2HPO_4{\cdot}2H_2O$, and NaCl were supplied by VWR (Leuven, Belgium).

2.2. Formulation of the dry powders

Dry powders were prepared by spray-drying as previously described, using amino acids (glycine, alanine, leucine, isoleucine), trehalose, DPPC and sulforhodamine 101 as a marker [10,17]. The fluorescent marker, sulforhodamine, was incorporated at a low load (0.2% w/w) in the formulations in order to allow quantification of the powder deposited in the MSLI (see below). The amino acids, trehalose and sulforhodamine were dissolved in 0.5 mM phosphate buffer, pH 7.4. DPPC was dissolved in 99.8% ethanol. The two solutions were combined to form a 70% ethanolic solution of 0.1% w/v total excipient concentration. The powders were produced using a LabPlant laboratory-scale spray-dryer (Lab-Plant Limited, Huddersfield, England) at low relative humidity (30–40%). The solutions were pumped into the drying chamber at a rate of 10 ml/min and pneumatically atomized through a two fluids external mixing 0.5 mm nozzle using compressed air at 0.5 bar. The inlet temperature was 100 °C; under these conditions, the outlet temperature varied between 52 and 62 °C. Each powder was formulated two to four times. Yields ranged between 5% and 15%. The powders were collected and stored in a dessicator (at 25% relative humidity and at room temperature).

2.3. Particle size, density and morphology

The primary volume median particle diameter (d) was measured by laser diffraction (HELOS, Sympatec, Clausthal-Zellerfeld, Germany). Powder samples were suspended in water in a 50 ml glass cuvette and stirred with a magnetic bar at 1000 rpm. A short period of sonication (30–60 s) at a power of 60 W (CUVETTE, Sympatec; 8.5 mm diameter ultrasound tip) was applied before sizing. A R2 lens allowing measurements in the range of 0.25–87.5 μm was used. The particle size analysis was performed by the WINDOX 3.4 software [20].

The powder density (ρ) was determined by tap density measurements, i.e., following 1000 taps which allowed the density to plateau [21].

The primary aerodynamic diameter of the particles, d_{aer}, was calculated based on the following definition: $d_{aer} = \sqrt{\rho/\rho_1}d$, with $\rho_1 = 1$ g/cm^3 [22].

The powder particles were viewed using a conventional scanning electron microscope (Phillips CM12/STEM, Eindhoven, Netherlands). The dry powder samples were mounted on metal grids and a 10-nm-thick gold film was sputter coated on the samples with a Balzers SCA 020 (Balzers Union, Liechtenstein) before visualization.

2.4. Aerosolization properties of the powders in vitro

The pulmonary deposition of the dry powders was estimated in vitro in a MSLI equipped with a USP induction port (Copley Scientific, Nottingham, UK), as previously described [18,23]. Twenty milliliters of water were poured into each of the four stages of the impinger to wet the collection surfaces. A hard gelatin capsule (size 2, Capsugel), previously stored in a dessicator for at least 2 days, was half-filled with the powder and placed in a Spinhaler® inhaler (Fisons, Bedford, MA). The capsule was then pierced and the liberated powder drawn through the impactor operated at 60 or 100 L/min. The use of a 60 L/min airflow rate allowed comparison of the aerodynamic behaviour of the albumin/trehalose/DPPC (30/10/60 w/w/w) powder with the albumin/lactose/DPPC (30/10/60 w/w/w) powder, previously prepared and analyzed at this airflow (Fig. 1) [10]. However, low resistance dry powder inhaler devices as the Spinhaler inhaler are best tested at 100 L/min (Figs. 2–4) [23–24]. The powder deposited on the four impinger levels was recovered by agitating the apparatus, removing the initial water and rinsing with additional fractions of water and ethanol up to reaching a total of 250 ml of a 60% ethanolic solution. The powder deposited in the throat and on the back filter was also collected. After dissolution of the particles, the fluorescence of each solution due to sulforhodamine was determined by spectrofluorimetry ($\lambda_{ex} = 586$ nm, $\lambda_{em} = 602$ nm). Each powder was analyzed twice in the MSLI.

The emitted dose was determined as the percent of total powder mass exiting the capsule. The cumulative mass of powder less than the stated size of each stage of the impactor was calculated and plotted on a log probability scale, as the percent of total mass recovered in the impactor against the effective cut-off diameter. The cut-off diameter of each individual stage (D) was determined as $D = D_{60}\sqrt{60/Q}$, where D_{60} is the cut-off diameter at a flow-rate of 60 L/min, i.e., 13.0, 6.8, 3.1 and 1.7 μm for stages 1 to 4, respectively, and Q is the flow-rate employed in the test [23]. The experimental mass median aerodynamic diameter (MMAD) of the particles was defined from this graph as the par-