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.

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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

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$_2$PO4·1H$_2$O, Na$_2$HPO$_4$·2H$_2$O, 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 ρ_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 (λ_{ex} = 586 nm, λ_{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-
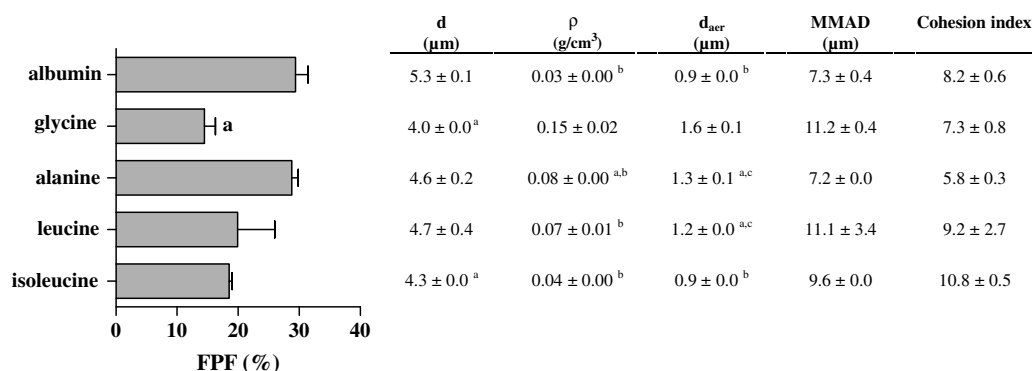
	d (µm)	ρ (g/cm³)	d_{aer} (µm)	MMAD (µm)	Cohesion index
albumin	5.3 ± 0.1	0.03 ± 0.00 [b]	0.9 ± 0.0 [b]	7.3 ± 0.4	8.2 ± 0.6
glycine	4.0 ± 0.0 [a]	0.15 ± 0.02	1.6 ± 0.1	11.2 ± 0.4	7.3 ± 0.8
alanine	4.6 ± 0.2	0.08 ± 0.00 [a,b]	1.3 ± 0.1 [a,c]	7.2 ± 0.0	5.8 ± 0.3
leucine	4.7 ± 0.4	0.07 ± 0.01 [b]	1.2 ± 0.0 [a,c]	11.1 ± 3.4	9.2 ± 2.7
isoleucine	4.3 ± 0.0 [a]	0.04 ± 0.00 [b]	0.9 ± 0.0 [b]	9.6 ± 0.0	10.8 ± 0.5

Fig. 1. Influence of the amino acid on dry powders physical and aerodynamic characteristics. The powders were formulated with 30% of albumin, glycine, alanine, leucine or isoleucine, 10% of trehalose and 60% of DPPC. The MSLI was performed at a flow-rate of 60 l/min. d, volume median particle diameter; ρ, the powder tap density; d_{aer}, the calculated primary aerodynamic diameter of the particles; MMAD, the mass median aerodynamic diameter of the particles measured experimentally. The cohesion index is the ratio of MMAD on d_{aer}. (a) $P < 0.05$ vs. albumin. (b) $P < 0.01$ vs. glycine. (c) $P < 0.05$ vs. isoleucine.

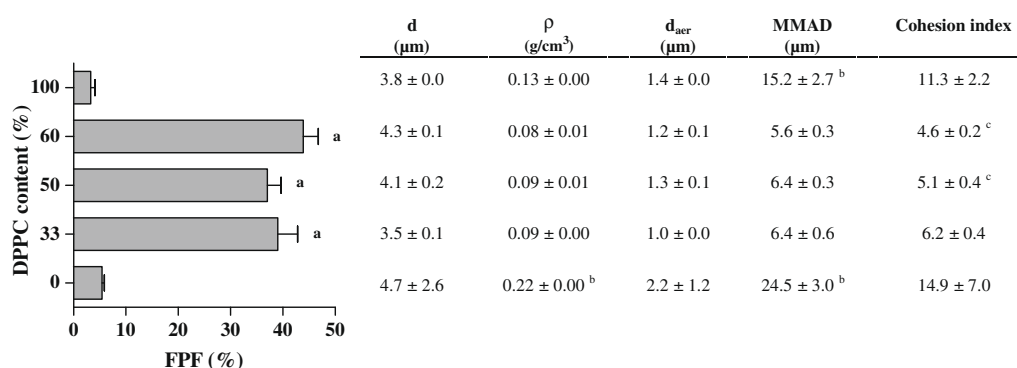|	d (µm)	ρ (g/cm³)	d_{aer} (µm)	MMAD (µm)	Cohesion index
100	3.8 ± 0.0	0.13 ± 0.00	1.4 ± 0.0	15.2 ± 2.7 [b]	11.3 ± 2.2
60	4.3 ± 0.1	0.08 ± 0.01	1.2 ± 0.1	5.6 ± 0.3	4.6 ± 0.2 [c]
50	4.1 ± 0.2	0.09 ± 0.01	1.3 ± 0.1	6.4 ± 0.3	5.1 ± 0.4 [c]
33	3.5 ± 0.1	0.09 ± 0.00	1.0 ± 0.0	6.4 ± 0.6	6.2 ± 0.4
0	4.7 ± 2.6	0.22 ± 0.00 [b]	2.2 ± 1.2	24.5 ± 3.0 [b]	14.9 ± 7.0

Fig. 2. Influence of DPPC proportion on dry powders physical and aerodynamic characteristics. DPPC was incorporated in variable content, and alanine and trehalose in equivalent amounts. The MSLI was performed at a flow-rate of 100 l/min. (a) $P < 0.01$ vs. DPPC 0% and DPPC 100%. (b) $P < 0.01$ vs. all other groups. (c) $P < 0.05$ vs. DPPC 0%. See Fig. 1 for definition of the acronyms.
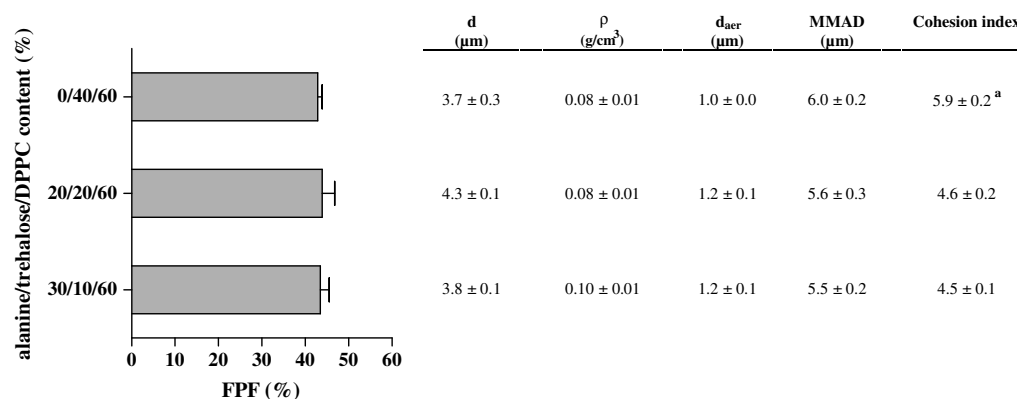

	d (µm)	ρ (g/cm³)	d_{aer} (µm)	MMAD (µm)	Cohesion index
0/40/60	3.7 ± 0.3	0.08 ± 0.01	1.0 ± 0.0	6.0 ± 0.2	5.9 ± 0.2 [a]
20/20/60	4.3 ± 0.1	0.08 ± 0.01	1.2 ± 0.1	5.6 ± 0.3	4.6 ± 0.2
30/10/60	3.8 ± 0.1	0.10 ± 0.01	1.2 ± 0.1	5.5 ± 0.2	4.5 ± 0.1

Fig. 3. Influence of the relative proportion of alanine and trehalose on dry powders physical and aerodynamic characteristics. DPPC was maintained at a proportion of 60%, and alanine and trehalose were incorporated in variable amounts. The MSLI was performed at a flow-rate of 100 l/min. (a) $P < 0.05$ vs. all other groups. See Fig. 1 for definition of the acronyms.

ticle size at which the line crossed the 50% mark. The fine particle fraction (FPF) was calculated by interpolation from the same plot as the fraction of powder emitted from the inhaler with an aerodynamic diameter ⩽5 µm [23].

The cohesion index was calculated by dividing the experimental MMAD on the computed d_{aer}. It is the ratio of the particle aerodynamic diameter measured experimentally, that is, the aerodynamic diameter of particle aggregates, on the calculated
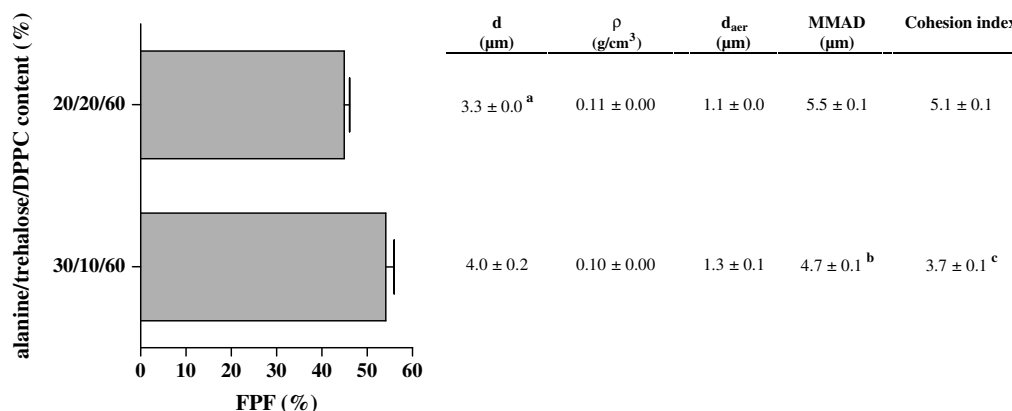
Fig. 4. Influence of the relative proportion of alanine and trehalose on dry powders physical and aerodynamic characteristics measured under strict relative humidity (RH) conditions. DPPC was maintained at a proportion of 60%, and alanine and trehalose were incorporated in variable amounts. The MSLI was performed at a flow-rate of 100 l/min. (a) $P < 0.01$ vs. 20/20/60 formulation (Fig. 3). (b) $P < 0.05$ vs. RH controlled 20/20/60 formulation. (c) $P < 0.05$ vs. RH controlled 20/20/60 formulation and 30/10/60 formulation (Fig. 3). See Fig. 1 for definition of the acronyms.

aerodynamic diameter of the individual particles. Therefore, it expresses the degree of particle aggregation [9].

2.5. Statistics

Values are expressed as means ± the standard error of the mean. Data were analyzed by using the GraphPad Prism version 4.00 software. Statistical analysis was made by one-way ANOVA with Tukey's multiple comparison post test.

3. Results and discussion

3.1. Trehalose instead of lactose and an amino acid instead of albumin

In the first step, powders were made of albumin/trehalose/DPPC or an amino acid/trehalose/DPPC in the proportion 30/10/60 by weight. Glycine, L-alanine, L-leucine and L-isoleucine were selected because they represent a certain range in hydrophobicity: glycine < alanine < leucine < isoleucine.

The albumin/trehalose/DPPC powder was less flowable than the albumin/lactose/DPPC powder previously prepared (Fig. 1) [10]. The emitted dose was 91% and the FPF 29% in the MSLI operated at 60 L/min. Both the trehalose and lactose powders had a primary volume median particle diameter around 5 μm and were extremely light (Fig. 1). The poorer behaviour of trehalose vs lactose in powder flow had previously been reported by others and us [9,16–17]. Multiple interrelated factors govern the aerodynamic behaviour of a dry powder: the particle size, the particle density, the surface properties, the components of the dry powder, the powder crystallinity, the powder hygroscopy. We had previously analyzed physico-chemical characteristics of the trehalose and lactose powders and we had not found any differences in overall morphology visualized by scanning electron microscopy [9], in chemical composition of the particle surface determined by X-ray photoelectron spectroscopy, in water content and hygroscopy measured by Karl Fisher titration and dynamic vapor sorption, respectively, and in powder crystallinity assessed by X-ray diffraction [17]. Additional information on these powders might be found using for instance atomic force microscopy (adhesion properties and particle surface topography) [25–26].

Using an amino acid instead of albumin affected aerosolization with no correlation with hydrophobicity (Fig. 1). The alanine and albumin dry powders had quite similar aerodynamic behaviour but the glycine, leucine and isoleucine powders were less respirable with FPF below 20% (Fig. 1). Replacing albumin with an amino acid increased powder tap density by up to fivefold (glycine) and slightly reduced volume median particle diameter. An increased density implies increased primary aerodynamic diameter (Fig. 1) and thereby increases the risk that particle aggregates become larger than 5 μm. Yet, increased density also implies decreased cohesion due to stronger separation forces due to gravity. The alanine/trehalose/DPPC dry powder presented the optimal balance between increased density that improves dispersibility (lowest cohesion index among all amino acids and albumin powders) and increases primary aerodynamic diameter (FPF as elevated as the albumin powder; Fig. 1). The larger the particle diameter, the higher the dispersibility. Yet, the nature of the excipient, albumin or amino acid, little affected the volume median particle diameter here (Fig. 1).

3.2. Optimal DPPC content

Having selected the alanine/trehalose/DPPC (30/10/60 w/w/w) powder, we then proceeded with the investigation of the optimal DPPC content. Good aerosolization performance was obtained for powders containing 33–60% of DPPC (89–95% emitted doses, 37–44% FPF at 100 L/min), but poor aerodynamic behaviour was observed for powders with 100% of DPPC or with no DPPC (94–97% emitted doses, 3–5% FPF; Fig. 2). The powder only made of DPPC was very cohesive probably because the gel phase of the phospholipid implies a certain particle softness and thereby an increased contact surface area between particles and high inter-particulate forces [27]. The increased density of the powder prepared without DPPC did not result in improved dispersibility; rather, cohesion increased (Fig. 2). DPPC, as a surfactant, is present at the air-liquid interface of the droplet during atomization. The presence of DPPC at the particle surface might decrease inter-particulate cohesion by reducing surface energy [17,28]. The hydrophobic character of DPPC also reduces moisture sorption and thereby cohesion [17]. Varying the relative proportion of DPPC from 0% to 100% did not significantly affect the volume median particle diameter (Fig. 2). In brief, DPPC and alanine/trehalose all appeared necessary to formulate free-flowing and respirable dry powders: DPPC improved powder dispersibility and the alanine/trehalose provided a rigid skeleton to the particle.

The powders containing 33–60% of DPPC showed a trend towards decreased MMAD and cohesion with increasing DPPC content (Fig. 2). This confirms that DPPC decreases aggregation between particles. Therefore, we came to the conclusion that maximal DPPC incorporation in combination with alanine and treha-

lose was best and we selected the powder containing 60% of DPPC as the optimal DPPC proportion.

3.3. Optimal alanine/trehalose relative proportion

We then investigated in which ratio the remaining 40% of the powder composition should be divided over alanine and trehalose. We formulated powders containing DPPC (60%) and 0/40%, 20/20% or 30/10% of alanine and trehalose. Trehalose was kept as excipient in all formulations because it stabilizes protein during drying and one of our goals is to incorporate proteins (therapeutic proteins or antigens) in the dry powder [17].

Varying alanine/trehalose proportions did not significantly affect the aerodynamic properties of the powders, neither their particle sizes and densities (Fig. 3). Yet, the powder prepared without alanine was significantly more cohesive when compared to the other blends. Additionally, the following trend could be distinguished: increasing alanine content slightly increased powder tap density and slightly decreased MMAD values and cohesion indexes (Fig. 3). The slight improvement in flowability provided by alanine might in part originate from the increase in powder density which could facilitate powder deagglomeration.

Alanine is a particularly good candidate excipient for inhalation dry powders. First, it allows the preparation of respirable powders with good dispersibility (Fig. 1) [14]. Second, it is able to stabilize proteins during thermal stresses and in solution [15]. Alanine is excluded from the protein surface and solute exclusion results in decreased protein unfolding and increased self-association of the native protein in order to decrease the protein surface area exposed to alanine [15].

3.4. Influence of humidity on aerosol performance

The previous parts of the work allowed selection of the dry powders made of alanine/trehalose/DPPC in the proportions 20/20/60 or 30/10/60 by weight as the most flowable dry powders. These powders were formulated in dry conditions (relative humidity between 30% and 40%), but their aerodynamic properties were assessed at room relative humidity and therefore under uncontrolled humidity conditions. Because dry powders equilibrate with the air relative humidity during handling, we repeated the evaluation of the aerodynamic properties of the selected dry powders at low relative humidity.

When assessed in dry conditions, the dry powder made of 30% alanine performed better than the 20% one in the MSLI. The MMAD value (4.7 µm vs 5.5 µm) and the cohesion index (3.7 vs 5.1) were smaller and the emitted dose (94% vs 92%) and FPF (54% vs 45%) were larger (Fig. 4). The relative humidity during aerosol evaluation clearly affected the aerodynamic behaviour of the alanine/trehalose/DPPC 30/10/60 powder (Fig. 3 vs. Fig. 4). The improvement in aerosol performance at low relative humidity was probably due to reduced moisture sorption, which diminished inter-particulate cohesion [1]. This highlights the importance of handling dry powders in a low relative humidity environment from their preparation up to their packaging in blisters.

3.5. Morphology of the alanine/trehalose/DPPC particles

The particles made of alanine/trehalose/DPPC (30/10/60 w/w/w) were visualized by electron microscopy and shown to be spherical in shape with a granular and rough surface (Fig. 5). The particles were put together as aggregates. Rugosity may limit point-to-point contacts between particles and reduce inter-particle cohesion forces, thereby contributing to the good powder dispersibility observed (Fig. 4) [13]. The granular appearance of the particle surface likely comes from alanine since particles only made of a sugar
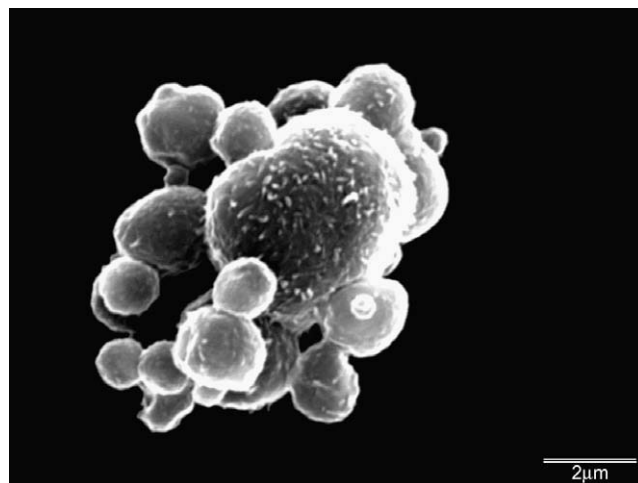

Fig. 5. Scanning electron microscope image of particles made with alanine/trehalose/DPPC (30/10/60, w/w/w).

and DPPC had a smooth surface [9]. The granulometry of the particles observed by electron microscopy agreed with the particle size measured by laser diffraction. Sizes varied between 1 and 5 µm in electron microscopy images (Fig. 5), and laser diffraction yielded an average volume median particle diameter of 4 µm (Figs. 1–4).

4. Conclusion

In this study, the aerodynamic behaviour of an inhalation dry powder was optimized by appropriately selecting powder composition. We confirmed that inter-particulate forces played a major role in the aerosilizaton properties of the powders and we reduced these forces by appropriately selecting excipients. Each excipient in the final formulation composed of alanine/trehalose/DPPC (30/10/60 w/w/w) helped to achieve optimal aerodynamic behaviour. This powder could incorporate small molecule drugs or biopharmaceuticals and be used to deliver these therapeutics to the lungs.

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