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Aerosolization properties, surface composition and physical state of spray-dried protein powders

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Abstract

Powder aerosols made of albumin, dipalmitoylphosphatidylcholine (DPPC) and a protein stabilizer (lactose, trehalose or mannitol) were prepared by spray-drying and analyzed for aerodynamic behavior, surface composition and physical state. The powders exited a Spinhaler™ inhaler as particle aggregates, the size of which depending on composition, spray-drying parameters and airflow rate. However, due to low bulk powder tap density (<0.15 g/cm^3), the aerodynamic size of a large fraction of aggregates remained respirable (<5 μm). Fine particle fractions ranged between 21% and 41% in an Andersen cascade impactor operated at 28.3 l/min, with mannitol and lactose providing the most cohesive and free-flowing powders, respectively. Particle surface analysis by X-ray photoelectron spectroscopy (XPS) revealed a surface enrichment with DPPC relative to albumin for powders prepared under certain spray-drying conditions. DPPC self-organized in a gel phase in the particle and no sugar or mannitol crystals were detected by X-ray diffraction. Water sorption isotherms showed that albumin protected lactose from moisture-induced crystallization. In conclusion, a proper combination of composition and spray-drying parameters allowed to obtain dry powders with elevated fine particle fractions (FPFs) and a physical environment favorable to protein stability.

Keywords: Dry powder aerosols; Spray-drying; Albumin; Dipalmitoylphosphatidylcholine; Fine particle fraction; X-ray photoelectron spectroscopy; X-ray diffraction; Water sorption

1. Introduction

In order to reach the lower respiratory tract and optimize systemic drug absorption, dry powder

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aerosols need to present aerodynamic diameters between 1 and 5 μm [1]. Larger particles impact in the oro-pharynx while sub-micron particles remain suspended in air and are exhaled [2]. Particle aggregation increases aerodynamic size and impedes efficient deep lung deposition of dry powders.

Conventional powder aerosols are produced by micronization of coarse particles in jet mills and present planar surfaces, geometric sizes <5 μm and particle densities of approximately 1 g/cm^3. Their small size as well as their irregular shape render them extremely difficult to disperse in dry powder inhalers [3]. Although the flowability of fine micronized powders can be improved by blending with coarse carrier particles [4], only 10–20% of nominal doses reach the deep lung [5].

We recently reported that spray-dried powders formed of GRAS excipients with an average geometric size of 5 μm achieved 40–60% fine particle fractions (FPFs) in cascade impactors in vitro, provided composition had properly been selected and bulk powder tap density was low (<0.15 g/cm^3) [6–8]. Small porous particles presented limited dependence of aerodynamic behavior with airflow rate [7,8]. They allowed the non-denaturing incorporation of interface sensitive human growth hormone as well as yielded high pulmonary bioavailabilities of this protein and of parathyroid hormone (1–34) following delivery to rats [8,9]. Elevated pulmonary absorption was partly due to permeation enhancer properties of dipalmitoylphosphatidylcholine (DPPC), the most abundant component of lung surfactant, that was used to prepare the aerosol powders [8,9].

The objective of this work was to further characterize small porous particles in terms of aerosolization properties, surface composition and physical state in order to determine which formulations could be the most suitable for pulmonary protein delivery. Dry powders were prepared with albumin, DPPC and lactose, trehalose or mannitol under variable spray-drying conditions. Aerodynamic behavior was estimated in vitro in an Andersen cascade impactor and by particle sizing at the exit of a Spinhaler™ inhaler. The chemical composition of the particle surface was determined by X-ray photoelectron spectroscopy (XPS) and the crystallinity, water content and hygroscopicity of the formulations were assessed by X-ray diffraction, Karl Fisher titration and dynamic vapor sorption, respectively.

2. Materials and methods

2.1. Chemicals

Human serum albumin (fraction V, 96–99% albumin), D-mannitol, D-trehalose dihydrate and 96% ethanol were purchased from Sigma (Saint-Louis, USA). α-Lactose monohydrate was obtained from Acros Organics (New Jersey, USA) and DPPC ($C_{40}O_8N_1P_1H_{80}$) from Lipoid (Lipoid, Ludwigshafen, Germany).

2.2. Formulation of the dry powders

Dry powders were formulated with DPPC, albumin and a sugar (lactose or trehalose) or a polyol (mannitol) by spray-drying [6]. Pure DPPC and albumin were also spray-dried to allow comparison of surface analysis data and water adsorption behaviors. DPPC was dissolved in 96% ethanol, and albumin and the sugar or polyol were dissolved in distilled water. The pH of the aqueous solution was adjusted to 7 by addition of a few droplets of NaOH 0.01 N (VWR International, Leuven, Belgium). The two solutions were combined to form a 70% ethanolic solution of 0.1% w/v total excipient concentration, except for powder ALD_c (Table 1) which was prepared from a 90% ethanolic solution of 0.25% w/v total excipient concentration.

Powders were produced using a Lab-Plant laboratory-scale spray-dryer (Lab-Plant, Huddersfield, England) at low relative humidity (RH <40%). Solutions were pumped into the drying chamber at a rate of 15 ml/min and pneumatically atomized through a two-fluid external mixing 0.5 mm nozzle using compressed air at 0.5 bar. The inlet temperature was established at 100 °C and in these conditions, the outlet temperature varied between 45 and 50 °C. Powders were stored in a dessicator at ambient temperature and 25% RH until analysis. Yields ranged between 10% and 20%.

Table 1
Composition, porosity and aerosolization properties[a] of the dry powders

Powder	Composition (wt.%)	d_{geo} (μm)[b]	ρ (g/cm^3)[c]	Surface area (m^2/g)[d]	d_{aer} (μm)[e]	ED (%)[f]	FPF (%)[g]	MMAD (μm)[h]
ALD	Albumin/lactose/DPPC (30/10/60)	4.9	0.058	9.7	1.18	83.8±0.6	37±2	6.6±0.3
ATD	Albumin/trehalose/DPPC (30/10/60)	4.8	0.058	6.6	1.16	89±2	28.5±0.2	6.8±0.2
AMD	Albumin/mannitol/DPPC (30/10/60)	4.5	0.025	6.5	0.71	84±12	21±4	10.2±0.2
ALD_c	Albumin/lactose/DPPC (30/10/60)	6.5	0.142	7.6	2.42	87±3	41±5	5.9±0.2
LD	Lactose/DPPC (40/60)	3.5	0.145	9.9	1.34	73±11	30±4	6.2±0.1

[a] Data are presented as the average of two or three measurements (±standard deviation); [b] Geometric particle diameter measured after dispersion by sonication; [c] Bulk powder tap density; [d] Specific surface area; [e] Computed primary aerodynamic diameter; [f] Emitted dose; [g] Fine particle fraction; [h] Mass median aerodynamic diameter.

2.3. Particle size, density and surface area

The primary geometric 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. We had previously validated particle sizing by laser diffraction following suspension in water and sonication [10]. The primary geometric particle diameter of powders similar in composition to those presented in this article was identical whether measured by microscopy, laser diffraction following dispersion with compressed air or laser diffraction following suspension in water and ultrasonic dispersion [10]. The geometric diameter of powder aggregates exiting a Spinhaler™ inhaler (Fisons, Bedford, MA) operated at a flow rate of 30 or 60 l/min was determined in the dry state (INHALER, Sympatec). An R2 lens allowing measurements in the range of 0.25–87.5 μm was used. The particle size analysis was performed by a WINDOX 3.4 software and the mass median particle diameter was considered. Measurements were performed in triplicate and were accurate up to the first decimal, except for mannitol powders released from the inhaler (variation over 3 μm).

The powder density (ρ) was determined by tap density measurements, i.e., following 1000 taps which allowed the density to plateau [11]. Assuming an efficient packing, the tap density of monodisperse spheres is approximately a 21% underestimate of the true particle density due to the void spaces between particles. Although polydispersity may reduce the void volume between particles, this is probably counterbalanced by an imperfect packing [12]. Measurements were performed in duplicate and were highly reproducible (up to the second decimal).

The primary aerodynamic diameter of the particles, d_{aer}, was calculated based on the following definition:

$$d_{aer} = \sqrt{\frac{\rho}{\rho_1}} d, \text{ where } \rho_1 = 1 \text{ g/cm}^3 \text{ [1].}$$

The surface area of the powders was determined in duplicate by nitrogen adsorption using the Brunauer–Emmett–Teller (BET) equation (Roquette Frères, Lestrem, France).

2.4. In vitro aerosol deposition

The pulmonary deposition of the dry powders was investigated in vitro using an Andersen cascade impactor (1 ACFM Eight Stage Non-Viable Cascade Impactor, Graseby Andersen, Atlanta, GA) under controlled relative humidity (30–40%). A hard gelatin capsule (size 2, Capsugel) previously stored in a dessicator for at least 2 days was filled with the powder to approximately 50% of its volume and placed in a Spinhaler™ inhaler. The capsule was pierced and the liberated powder drawn through the impactor operated at 28.3 l/min for 10 s [13]. The mass of powder deposited on each stage was determined by measuring the weight difference of glass fiber collection discs (Graseby Andersen) placed on the impactor trays.

The emitted dose was defined as the percent of total powder mass exiting the inhaler. The cumulative mass of powder less than the stated size was calculated and plotted on a logarithmic scale, as percent of total mass recovered in the Andersen impactor against the effective cut-off diameter. The experimental mass median aerodynamic diameter (MMAD) of the particles was defined on this graph as the particle size at which the line crossed the 50% mark. The geometric standard deviation (GSD) was determined as $GSD = \sqrt{\frac{SizeX}{SizeY}}$, where sizes X and Y are particle sizes for which the line crosses the 84% and 16% mark, respectively. GSD ranged between 1.7 and 2.4. The FPF was calculated from the same plot as the fraction of powder emitted from the inhaler with an aerodynamic size ≤5 μm [13]. Measurements were performed in duplicate.

2.5. Surface analysis

The surface composition of the dry powder aerosols was analyzed by XPS. This technique provides a direct chemical analysis of solid surfaces on a depth of approximately 5 nm [14]. Briefly, powder samples are irradiated by an X-ray beam, which induces the ejection of electrons from the atoms. The kinetic energy of the photoelectrons is analyzed and their binding energy is determined. Since the binding energy of electrons in the atom of origin is characteristic of the element and affected by its chemical environment, the method provides an elemental analysis and further information on functional groups.

The powders were gently compacted with a spatula into small stainless-steel troughs of 4 mm inner diameter and 0.5 mm depth. A polyacetal cylinder (Delrin), cleaned with isopropanol, was placed above the trough and pressed to obtain a smooth surface. XPS analyses were performed with a Kratos Axis Ultra spectrometer (Kratos Analytical, Manchester, UK) equipped with a monochromatized aluminium X-ray source (powered at 10 mA and 15 kV) and an eight-channeltron detector. The spectrometer was interfaced with a Sun Ultra 5 workstation for instrument control, data acquisition and processing. The angle between the normal to the sample surface and the lens axis was 0°. The analyzed area was 700×300 μm. The constant pass energy of the analyzer was set at 160 eV for the survey spectrum and 40 eV for detailed peaks; the hybrid lens mode was used. The following sequence of spectra was recorded: survey spectrum, C_{1s}, O_{1s}, N_{1s}, P_{2p} and C_{1s} again to check the stability of charge compensation and the absence of degradation of the sample during analyses.

Molar concentration ratios of elements were calculated using peak areas normalized on the basis of acquisition parameters, sensitivity factors provided by the manufacturer (based on experimental Wagner sensitivity factors) and transmission factors included in the software (depending on kinetic energy, analyzer pass energy and lenses combination). The C_{1s}, O_{1s} and N_{1s} peaks were decomposed with the least squares fitting routine provided in the manufacturer Unix based Vision 2.1.2. software with a Gaussian/Lorentzian ratio of 70/30 and after subtraction of a linear baseline, according to a method described previously [15,16]. The surface analysis was performed in duplicate on two different powder batches.

2.6. X-ray diffraction

The crystallinity of the dry powder aerosols was assessed by X-ray diffraction. Powder patterns were acquired at ambient temperature and atmosphere on a computer-controlled Philips PW1710 diffractometer equipped with a PW3710 mpd control unit, a monochromator (Cu Kα radiation tube operated at 40 kV, 30 mA) and a scintillation counter. Data were collected on two different powder batches over an angular range from 4° to 50° in 2θ in continuous scan mode using a scan speed of 0.02 2θ/s.

2.7. Water content

The water content of the dry powders was assessed by Karl Fisher titration in dry methanol (Hydranal®, Riedel-de Haën, Seelze, Germany) using a DL35 titrator (Mettler-Toledo, Greifensee, Switzerland). Sample mass was approximately 20 mg and Hydranal® composite 1 (Riedel-de Haën) was used as the titration reagent. Measurements were performed in triplicate.

2.8. Gravimetric sorption analysis

The hygroscopicity of the dry powders and their stability at high RH was evaluated by determining water sorption isotherms (n=1). A dynamic vapor sorption instruments DVS-100 (Surface Measurement Systems, London, UK) instrumented with a Cahn D200 microbalance was used. Samples were weighted into glass sample holders and loaded rapidly into the unit. The powder sample was then equilibrated (minor changes in sample weight over 5 min) with dry air, and then equilibrated sequentially at increasing RH up to 90%. A ramp with increments of 10% RH every 3 h was used.

2.9. Statistics

Statistical differences between fine particle fractions (n=2), particle sizes (n=2) and powder water contents (n=3) were demonstrated by one-way analysis of variance (ANOVA) and Tukey tests using the software Sigmastat for Windows (SPPS, San Rafael, CA, USA).

3. Results and discussion

3.1. Composition, porosity and aerosolization properties of the dry powders

Dry powders were prepared with a combination of two or three of the following excipients: albumin, lactose, trehalose, mannitol and DPPC. Table 1 details the composition, the primary geometric particle diameter, the bulk powder tap density, the specific surface area, the computed primary aerodynamic diameter, the emitted dose, the FPF and the MMAD of the main powder aerosols described in this article. All powders were produced using identical formulation and spray-drying parameters, except for powder ALD_c which was prepared from a feed solution more concentrated in ethanol and excipients.

The particles produced were small and porous, with primary geometric particle diameters between 3.5 and 6.5 μm and bulk powder densities <0.15 g/cm^3. The porous character of the dry powders was substantiated by the high values of specific surface areas: full spheres of 5 μm and 1 g/cm^3 theoretically present specific surface areas of 1 m^2/g, while experimental values reached 10 m^2/g (Table 1). Scanning electron microscopy images showed hollow particles with smooth or wrinkled surfaces [6,7,10].

Particle sizing in an Andersen cascade impactor and by laser diffraction indicated that powder aerosols exited a Spinhaler™ inhaler device as particle aggregates rather than isolated particles (Table 1; Fig. 1). Computed primary aerodynamic diameters ranged between 0.7 and 2.4 μm, whereas MMAD measured experimentally in the impactor operated at 28.3 l/min were 2.5- to 14.4-fold larger (Table 1). Geometric sizes of particles exiting the Spinhaler™ device were measured by laser diffraction as well; sizes at an operating flow rate of 30 l/min were 1.8- to 7.3-fold larger than primary geometric particle diameters (Fig. 1). It is noteworthy that aggregates released from the inhaler appeared larger in the Andersen impactor than in the laser diffraction system, probably reflecting differences in apparatus configuration, functioning and method of particle sizing [12]. Aerodynamic sizes of large porous aerosol particles were similarly reported to be twice larger when measured with an Andersen impactor than with an Aerosizer™ [12]. Interestingly, Dunbar et al. [17]

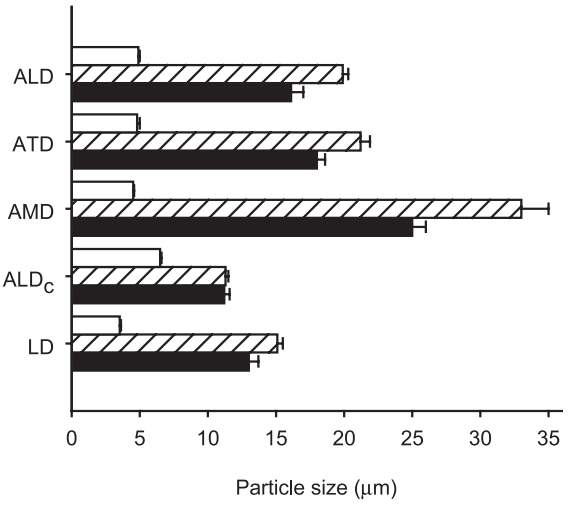

Fig. 1. Comparison between (open bars) primary geometric particle diameter and the size of particle aggregates released from a Spinhaler™ inhaler at (open bars with dark slashmarks) 30 or (dark shaded bars) 60 l/min. Data are presented as the mean of three measurements. Error bars are standard deviations.

showed that the MMAD of large porous particles estimated in a multi-stage liquid impinger predicted well the in vivo deposition of the aerosols in healthy subjects, in support of data collected in impactors.

Doses emitted from the Spinhaler™ inhaler ranged between 70% and 90% of nominal doses and FPFs reached up to 40% (Table 1). Those values were high, especially given the sub-optimal inhaler and airflow rate employed [4]. They were comparable to those previously obtained for similar small porous aerosols [6,7] as well as large porous particles [12]. Although particles exited the Spinhaler™ inhaler as aggregates, low powder densities resulted in a large fraction of particle aggregates with an aerodynamic size <5 μm (Table 1).

As reported previously, replacing lactose by trehalose or mannitol as well as removing albumin from the formulation decreased the fine particle fraction (Table 1; $p<0.05$) [6,18]. Increasing total excipients and ethanol concentrations of the feed solution tripled bulk powder tap density and slightly increased primary particle diameter (Powder ALD_c in Table 1) [6]. The aerodynamic behavior of a powder aerosol depends on multiple interrelated factors as primary particle size, particle density, powder composition, powder crystallinity, surface properties and powder cohesiveness [6,19]. Mannitol rendered the powder highly cohesive and thereby increased the size of particle aggregates exiting the inhaler and decreased the fine particle fraction (Powder AMD in Fig. 1 and Table 1) [6,10]. Powder ALD_c had a similar FPF as powder ALD, even though its primary aerodynamic diameter was twice larger (Table 1). These similar depositions in vitro resulted from increased deaggregation of powder ALD_c that partly followed from its larger geometric particle size (Fig. 1; Table 1) [20]. Greater deaggregation also improved constancy in aerosolization properties since powder ALD_c was the only one that showed no change in aggregate size with an increase in airflow rate (Fig. 1; $p>0.05$).

3.2. Surface analysis

Proteins can adsorb at the air–liquid interface of droplets in spray, unfold and aggregate at the droplet surface [21,22]. Surfactants have been shown to reduce this phenomenon by excluding the protein from the interface [21,22]. Chemical composition of particle surface also governs interparticulate forces that influence dispersion of powder aerosols during inhalation [3]. Analysis of surface composition of spray-dried protein powders may therefore help choosing formulation and spray-drying parameters favorable to protein integrity and good aerosolization performance. We employed XPS to determine the surface composition of the dry powders described in Table 1 as well as of a powder made of albumin/DPPC 40/60 w/w (powder AD) in order to further interpret the data.

As a first step, pure spray-dried DPPC and albumin were analyzed. Table 2 presents the concentration ratios of different elements: carbon in different chemical forms, oxygen, phosphorus, protonated (N_{pr}) and unprotonated nitrogen (N_{upr}). Measured ratios were in fair agreement with those expected from stoichiometry. However, the concentration of carbon bound to carbon and hydrogen (C−(C,H)) was higher than expected. This may be due to a surface contamination by hydrocarbonaceous compounds from the atmosphere [23] or, for DPPC, to an orientation of the aliphatic chains to the air phase. This latter assumption is supported by the $(N/C)_{xps}$ ratio of pure DPPC particles (0.016) which was lower than the $(N/C)_{sto}$ ratio (0.025) and identical to that found for pure DPPC monolayers orientated on mica [24]. The carbon enrichment of pure DPPC and albumin particle surfaces limits the use of this element to examine the surface composition of dry powder formulations.

Table 2
XPS analysis of pure DPPC and albumin after spray-drying

Atom type (Z)	DPPC		Albumin	
	$(Z/N)_{sto}$ [a]	$(Z/N)_{xps}$ [b]	$(Z/N_{upr})_{sto}$ [a]	$(Z/N_{upr})_{xps}$ [b]
Ctot	40	65±3	4.69	5.8±0.3
C−(C,H)	30	52±2	2.12	3.12±0.07
C−(O,N)	8	10±2	1.41	1.50±0.02
C=O, O−C−O	–	–	1.00	1.05±0.05
COOR	2	2.8	0.16	0.16
O	8	8.9±0.6	1.43	1.27±0.04
P	1	1.194±0.008	0	–
N_{pr}	1	1	0.26	0.035±0.001

N_{upr}=unprotonated nitrogen, N_{pr}=protonated nitrogen.
[a] Ratios calculated based on stoichiometry; [b] Experimental ratios, ± standard deviation.

XPS analysis was then used to quantify the concentration of markers specific for DPPC and albumin in the dry powders. Phosphorus is characteristic of DPPC. Protonated nitrogen (N_{pr}) is found in choline moieties of DPPC and in lateral chains of amino acids. N_{pr} was tentatively investigated as a second marker for the phospholipid. Fig. 2a plots the ratio $(N_{pr}/C)_{xps}$ versus $(P/C)_{xps}$ for all formulations investigated. The 1:1 relationship is in good agreement with the stoichiometry of DPPC. The contribution of albumin in total N_{pr} content appeared therefore negligible. $(N_{pr}/C)_{xps}$ for albumin was

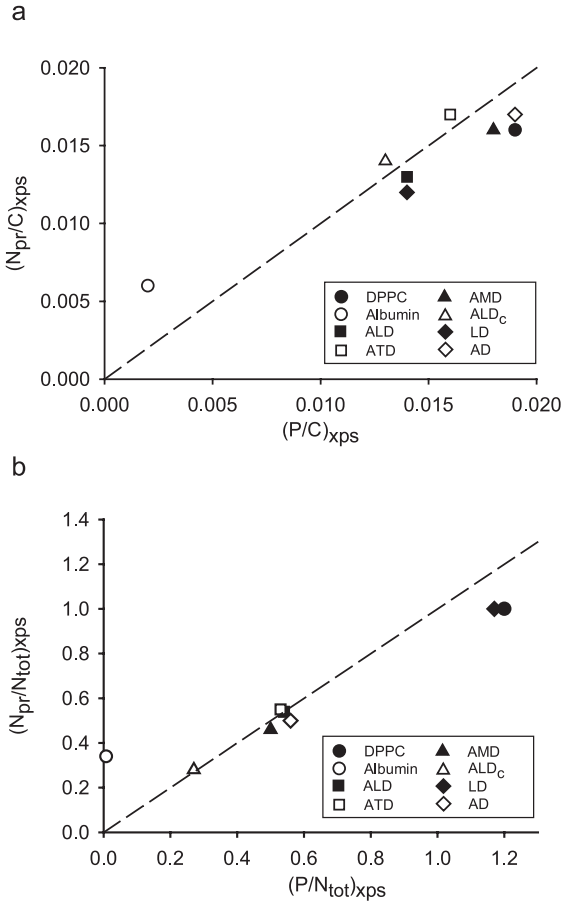

Fig. 2. Comparison of elemental concentration ratios determined by X-ray photoelectron spectroscopy: (a) (N_{pr}/C) vs. (P/C) and (b) (N_{pr}/N_{tot}) vs. (P/N_{tot}). The mean of two or three measurements is presented for the powders described in Table 1, spray-dried pure DPPC and albumin as well as a powder made of albumin/DPPC 40/60 w/w (powder AD). Error bars are not shown for clarity. The dotted line represents the 1:1 relationship.

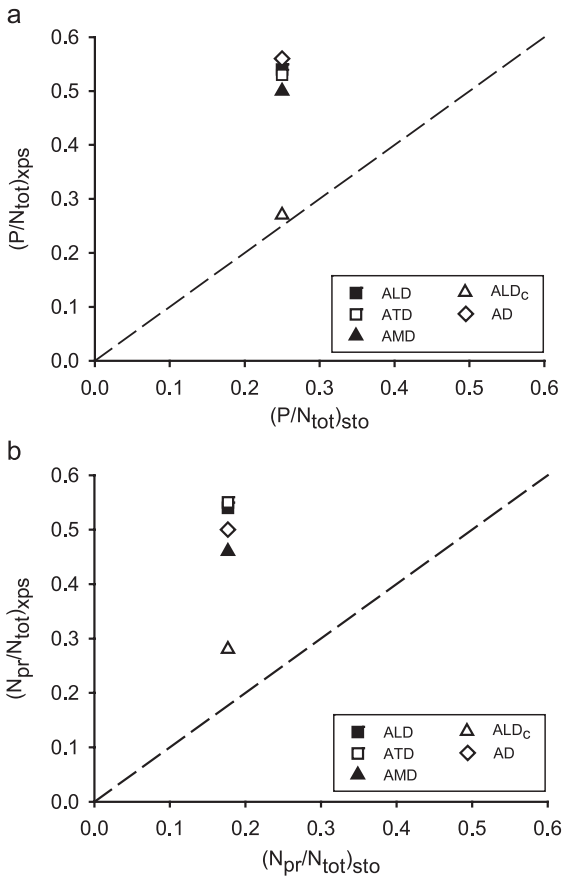

Fig. 3. Comparison between (a) (P/N_{tot}) ratio determined by X-ray photoelectron spectroscopy (XPS) and that based on stoichiometry and formulation; (b) (N_{pr}/N_{tot}) ratio determined by XPS and that expected from the stoichiometry of DPPC, $(N_{pr}/N_{tot})_{xps}$ of albumin and the formulation. The data from powders ALD, ATD, AMD, ALD_c (see Table 1 for powder description) and a powder made of albumin/DPPC 40/60 w/w (powder AD) are presented (mean of two or three determinations). Error bars are not shown for clarity. The dotted line represents the 1:1 relationship.

equal to 0.006, whereas the stoichiometric ratio would be 0.2 if lateral chains were protonated. A similar result was obtained for $(N_{pr}/N_{upr})_{xps}$ and $(N_{pr}/N_{upr})_{sto}$, with values of 0.035 and 0.26, respectively (Table 2). This confirms that most nitrogen atoms in albumin were unprotonated and fits with the pH of the feed solution higher than the isoelectric point of albumin (4.8). Consistently, Fig. 2b shows a 1:1 relationship between $(N_{pr}/N_{tot})_{xps}$ and $(P/N_{tot})_{xps}$.

In Fig. 3, $(P/N_{tot})_{xps}$ and $(N_{pr}/N_{tot})_{xps}$ are respectively compared with $(P/N_{tot})_{sto}$ and $(N_{pr}/N_{tot})_{sto}$, the ratios expected from the stoichiometry of excipients

and formulations. An enrichment of the particle surface with DPPC was observed for powders ALD, ATD, AMD and AD. Adler et al. [21] showed that polysorbate 80 and sodium dodecyl sulfate, when added in high concentrations in the bulk solution, excluded bovine serum albumin from the surface of spray-dried particles. Reduction of protein adsorption at the air–liquid interface of droplets by DPPC might improve protein integrity after spray-drying due to avoidance of surface denaturation [22]. Consistently, the proportion of human growth hormone aggregates was decreased from 3.9% to <1% following spray-drying when adding DPPC to the feed solution [8]. DPPC-enriched surfaces are hydrophobic, which might result in lower interparticulate forces due to reduced surface hygroscopicity and therefore improved aerosol performance [19]. Surface composition was not altered by incorporating lactose, trehalose or mannitol in the preparation and could not explain the differences in aerosolization of powders ALD, ATD and AMD and, in particular, the increased cohesion of powder AMD (Figs. 1 and 3; Table 1).

The surface of powder ALD_c did not show an enrichment of DPPC relative to albumin (Fig. 3). Concentrated solutions might have prevented DPPC to move freely towards the air/liquid interface during droplet formation or albumin to diffuse from the edge of the droplet to its center during drying. DPPC might also have precipitated less readily at the droplet surface, compared to albumin, due to increased solubility in the ethanol concentrated feed solution [25].

In the case of powder LD, the $(O/P)_{xps}$ ratio was compared to the $(O/P)_{sto}$ ratio. They were respectively 15 ± 2 and 14, showing that the surface composition of the powder free of albumin was similar to the bulk composition. Surprisingly, DPPC hindered the migration of surface-active albumin towards the particle surface and did not do so for lactose which has no surface activity [21]. However, sugars are known to interact with polar heads of DPPC and this interaction might decrease DPPC availability for surface migration [26]. Another hypothesis is that the fourfold increase in lactose concentration in the feed solution accelerated its precipitation at the droplet surface during the drying process [25].

3.3. Crystallinity, water content and hygroscopicity

A crystalline state of excipients is detrimental for protein stability because of phase separation and loss of excipient–protein interaction [27]. The physical state of a powder aerosol can also affect aerosolization properties [28,29]. Therefore, we analyzed the crystallinity of the dry powders described in Table 1 by X-ray diffraction. The diagrams revealed the characteristic peak of the gel phase of DPPC at approximately $2\theta=21^\circ$ [30], whatever the sugar or polyol incorporated, the presence or absence of albumin as well as the spray-drying conditions (Fig. 4 and data not shown). The organization of DPPC further supports the orientation of its aliphatic chains to the air phase and suggests a phase separation of DPPC from the protein and sugar/polyol. No sugar or mannitol crystals were observed in any formulation (Fig. 4 and data not shown). However, mannitol crystallizes more easily upon lyophilization and spray-drying than lactose or trehalose and X-ray diffraction might not be sensitive enough to detect mannitol crystals in the highly organized DPPC structure [18,28].

Because the amount of free water in a powder influences its physical stability and controls the magnitude of capillary forces that hold particles in aggregates [3], we measured residual moisture contents by Karl Fisher titration and investigated water sorption behavior. Water contents were between 5.0% and 6.5% ($p>0.05$), except for powder LD which was slightly dryer (4.6%; $p<0.05$) as expected due to the absence of the protein [31]. It has been determined that the monolayer moisture content of different lyophilized protein/sugar mixtures [31], methionyl human growth hormone [32] and tissue type plasminogen activator (tPA) [32] ranged between 4.5% and 7%, and that drying freeze-dried tPA to below its monolayer water level had detrimental effect on its physical stability [32]. All the powders containing albumin showed a water sorption isotherm slightly below that of spray-dried pure albumin and significantly above that of spray-dried pure DPPC (Fig. 5). This indicates that the protein content controlled the behavior in water sorption isotherm [31]. In the absence of protein in the formulation, lactose crystallized with subsequent release of entrapped water at 50% relative humidity (Fig. 5), suggesting that the

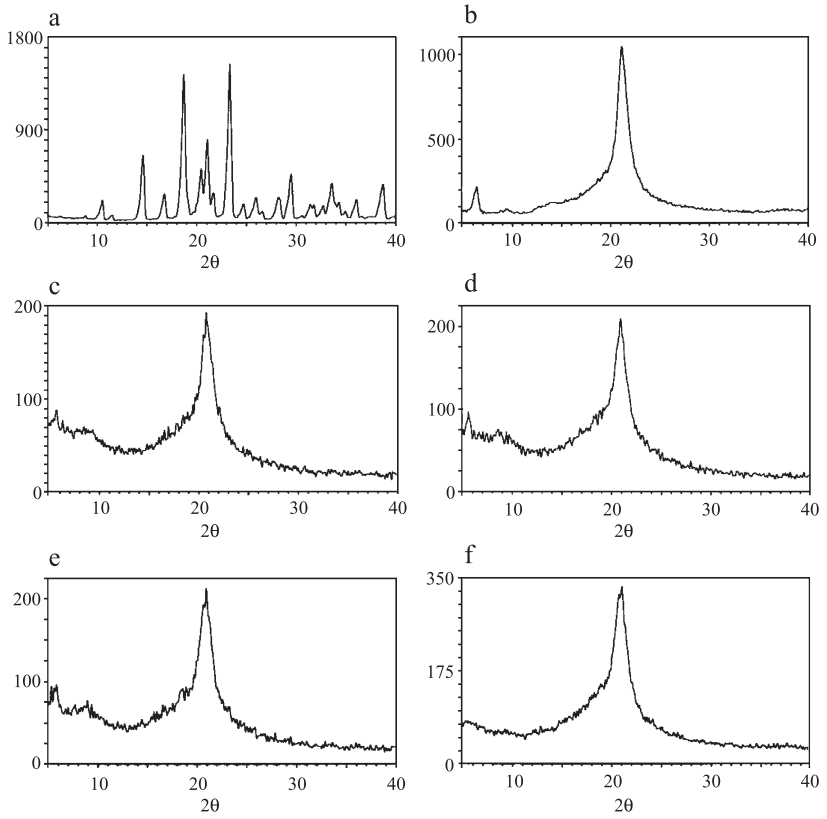

Fig. 4. X-ray diffraction diagrams of (a) spray-dried pure mannitol; (b) spray-dried pure DPPC; (c) powder ALD; (d) powder ALD_c; (e) powder AMD; (f) powder LD (see Table 1 for powder description). The diagram of powder ATD was similar to that of powder ALD or AMD and is not shown.

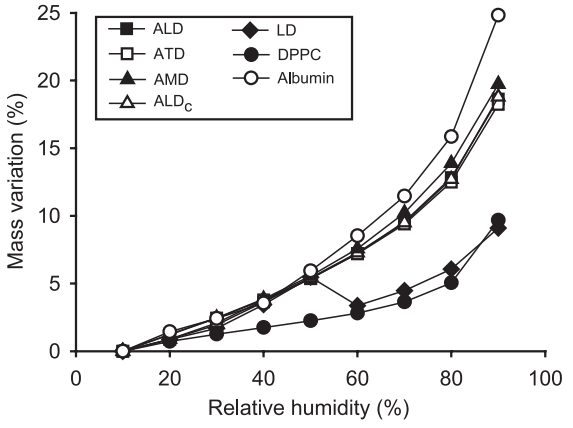

Fig. 5. Water adsorption isotherms of powders described in Table 1 as well as of spray-dried pure DPPC and albumin.

powders made of lactose were essentially amorphous upon spray-drying [31]. Proteins with a high molecular weight have previously been demonstrated to protect sugars from crystallization at high relative humidity in protein/sugar lyophilized powders [31]. Because powders ALD, ATD, AMD and ALD_c contained the same amount of water and took up water similarly in gravimetric sorption studies, capillary forces were not involved in the differences in aerodynamic behavior and cohesion observed between them (Table 1).

4. Conclusion

In this article, we characterized spray-dried powder aerosols formed of albumin, DPPC and a sugar or a polyol in order to help defining composition and spray-drying parameters that could be the most

interesting for protein delivery to the lungs. Particles prepared with lactose or trehalose were small, porous and highly respirable even though they behaved as particle aggregates. DPPC had a large surface excess relative to albumin under certain spray-drying conditions and self-organized in a gel phase in the particle. No sugar or mannitol crystals could be detected and albumin/sugar/DPPC combinations were not prone to crystallization upon exposure to moisture. The surface enrichment of DPPC relative to the protein and the avoidance of crystallization offer a physical environment favorable to protein stability. It remains to be determined if the formulation matrix is physically stable upon long-term storage as well as if it effectively protects therapeutic peptides and proteins against degradation.

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