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Research paper

Optimisation of spray-drying process variables for dry powder inhalation (DPI) formulations of corticosteroid/cyclodextrin inclusion complexes

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ABSTRACT

This study aims to develop and characterise a beclomethasone diproprionate:γ-cyclodextrin (BDP:γ-CYD) complex and to optimise the variables on the spray-drying process, in order to obtain a powder with the most suitable characteristics for lung delivery. The spray-dried powder – in a mass ratio of 2:5 (BDP:γ-CYD) – was physically mixed with three carriers of different particle sizes and in different ratios.

Particle-size distribution, shape and morphology, moisture content, and uniformity in BDP content of formulations were studied. *In vitro* aerolisation behaviour of the formulations was evaluated using the Rotahaler, and the performance was characterised based on the uniformity of emitted dose and aerodynamic particle-size distribution (respirable fraction (RF), as a percentage of nominal dose (RFN) and emitted dose (RFE)).

The most suitable conditions for the preparation of BDP:γ-CYD complexes were obtained with the solution flow of 5 ml/min, T_{in} of 70 °C and T_{out} of 50 °C.

Statistically significant differences in the aerodynamic performances were obtained for formulations containing BDP:γ-CYD complexes prepared using different solution flows and different T_{in} ($p < 0.05$). RFN and RFE vary in direct proportion with T_{in}, while an inverse relationship was observed for the solution flow. A direct correlation between the RFE and the T_{out} was identified.

Performance of the formulations was compared with an established commercial product (Beclotaide Rotacaps 100 μg) with improved performance of RF: formulations with respitose carrier attained RFN and RFE twofold greater, and formulations based on 63–90 μm fraction lactose and trehalose achieved a threefold improvement; also, all formulations showed that the percentage of dose of BDP deposited in the "oropharynx" compartment was reduced to half.

1. Introduction

The pulmonary administration of corticosteroids, such as beclomethasone diproprionate (BDP), has shown to be very efficient in asthma therapy and prophylaxis of its acute crises [1–3].

The pulmonary administration of drugs in the form of complexes with cyclodextrins (CYDs) is believed to present advantages through the improvement of the local effect of drug via increased stability and solubility [4,5].

Spray-drying is not only a process that can be used for the preparation of CYD complexes, but also a widely used method for the preparation of microparticle powders to be used as dry powder inhalers [6,7]. The particles obtained via spray-drying are usually spherical, with a small diameter (1–5 μm) and narrow particle-size distribution [8–10]. These characteristics and the aerodynamic properties of the powders obtained are influenced by the nozzle, the viscosity of the feeding solution, and the outlet temperature (T_{out}), the latter being dependent on the two spray-drying process variables: inlet temperature (T_{in}) and solution flow [11–13].

The cohesive nature of these micron-sized particles may result in poor flow characteristics, which are problematic in powder manufacturing and filling processes. To improve handling, powders are typically mixed with carrier powder particles of larger size, and for efficient lung deposition, powders of smaller particle size must subsequently be dispersed from the carrier by shear forces during inhalation [12].

Complex formation between BDP and CYDs has previously been reported elsewhere [14,15] using solubility phase tests to prove the formation of stable complexes in the following order of affinity: γ-CYD > DM-β-CYD > HP-β-CYD > β-CYD. DSC, IR and X-ray analyses were used to provide evidence of complex formation [14,15]. Thus, in the current studies, γ-CYD was selected for the preparation of the complex with BDP as it forms complexes with the highest K_s values ($K_s = 2110\ M^{-1}$) [14,15] and it does not pres-

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ent the toxicity of DM-β-CYD [16]. The complex formation of γ-CYD can be favoured for the biggest flexibility of the structure of this CYD, when compared with the others [17]. The advantage of using BDP in the complex form is also justified as it prevents the drug molecule association. The complex form improves the homogeneity of the drug into formulation and facilitates its preparation.

Some authors suggest that complex formation of CYDs can also increase the drug absorption at the pulmonary level and improve the lung bioavailability [5,18,19]. It has also been reported [20] that the use of DM-β-CYD with a lipid–polycation–pDNA leads to improvement in dispersibility, enhancement of aerosolisation properties and increase in the biological functionality. CYDs may be used in inhalation powders to improve pharmaceutical and biopharmaceutical properties of drugs without lowering their pulmonary deposition. The *in vitro* pulmonary depositions of budesonide and budesonide:γ-CYD complexes were determined from the Taifun multi-dose dry powder inhalers [21], while that of budesonide:DM-β-CD complexes were determined from Flow-Caps® [6]. Evrard et al. [22] have demonstrated safety on short-term exposure of inhaled HP-β-CYD, γ-CYD and randomly methylated β-CYD formulations in terms of lung and renal integrity and function, as those CYDs were non-toxic after assessing bronchoalveolar lavage, lung and kidney histology, bronchial responsiveness to methacholine and blood urea.

2. Materials and methods

2.1. Materials

BDP was a generous gift from Hovione Sociedade Química SA, and γ-CYD was a kind gift from Wacker-Chemie GmbH. Monohydrated lactose (Granulac 70) was purchased from Meggle, dehydrated trehalose was purchased from Sigma, and Respitose was donated by DMV International Pharma. The lactose and trehalose were sieved for 20 min using a sieve shaker AS 200 Digit, Retsch, collecting the size fraction 63–90 μm.

The solvents used were absolute ethanol (pa, Merck) and demineralised water. All other reagents were of analytical grade.

The Rotahaler was selected as a model inhalation system for the evaluation of aerodynamic characteristics of the formulations. White capsules of hydroxypropylmethylcellulose (HPMC), number 3, from Shionogi Qualicaps, were used.

Commercial product selected for comparative evaluation was originator BDP product – Beclotaide Rotacaps 100 μg (batch 10341091).

2.2. Methods

2.2.1. Assay for BDP

An assay method for BDP was developed and validated based on UV spectroscopy (spectrophotometer HITACHI, Model U2000) using a wavelength of 238 nm. This method was established to be linear, accurate, precise and selective for the measurement of BDP in the matrix of the prepared formulations.

2.2.2. Selection of the best formulation and of the best spray-drying conditions

A complex from BDP and γ-CYD (BDP:γ-CYD) was prepared by spray-drying in order to choose the best carrier and the best ratio of complex to carrier for lung delivery formulations.

The selection of the best formulation was based on the following:

- Preparation of the complex: spray-drying was performed using a laboratory-scale spray-dryer, model B-191, Buchi, with a solution containing 0.7% (w/v) of BDP and 1.8% (w/v) of γ-CYD in ethanol/water (75:25), corresponding to a mass ratio of (2:5) BDP:γ-CYD. After heating at 60 °C and cooling to 50 °C, the solutions were atomised. The following conditions were used: flow rate = 5 ml/min, T_{in} = 70 °C, corresponding to a T_{out} of 48 °C, compressed air at 600 l/h and the aspirator at 31.5 m^3/h.
- Physical mixture with the carrier: the complex was uniformly mixed, for 4 h, in a rotating mixer Fisher–Kendall model 12-811, with either the 63–90 μm fraction of lactose or respitose or trehalose obtained by sieving (93 and 193 parts) (Table 1). The mixture time was defined by the formulation 1Lac, through the assay of uniformity of content in BDP, carried out after every hour of mixing, by collecting five samples, at different points of time during the formation of the mixture and its dissolution in ethanol/water (75:25).

Selection of the best spray-drying conditions.

- Solutions to be submitted to spray-drying were prepared as above. A fractional design was employed to evaluate the influence of solution flow rate and T_{in}. The solutions were atomised at three different flow rates (5, 8 and 11 ml/min) using fixed values for compressed air (600 l/h) and aspirator (31.5 m^3/h). Two T_{in} values were used: 50 °C and 70 °C. After spray-drying, each resulting powder was collected by cyclone separation and transferred to glass vials.

2.2.3. Powder characterisation

Particle-size distribution of the spray-dried complex and the different mixtures with the carriers was determined using an Aerosizer® LD.

The morphologic properties of the spray-dried powders were characterised by scanning electronic microscopy (JEOL, Model JSM.5200LV).

2.2.4. Capsules filling

Capsules were manually filled, to a fill weight equivalent to a nominal dose of 200 μg of BDP, i.e., for the formulations (2:5):93 (corresponding to 2% w/w BDP) and (2:5):193 (corresponding to 1% w/w BDP); each capsule contains 10 ± 1 mg and 20 ± 1 mg of powder mixture, respectively.

2.2.5. Aerodynamic characteristics and aerosol performance

The aerodynamic characteristics and the aerosol performance of the formulations were tested by an impaction-based apparatus – Apparatus A (Glass Impinger), also known as a twin stage liquid impinger (TSLI) or simply twin impinger (TI), at a flow rate of 60 ± 5 l/min [23].

Prior to use, stage 1 and stage 2 of TI were charged with 7 and 30 ml of ethanol/water (75:25), respectively. A single-dose dry powder inhalation device, the Rotahaler, was used to evaluate the aerosol performance of the formulations.

Each determination consisted of discharge of the contents of five capsules with three replicate determinations made for each formulation. The BDP content of each compartment (upper, U = throat; medium, M = stage 1; and lower, L = stage 2) and the remaining in the capsule were determined as described in the literature [24].

Emitted fraction (percentage of BDP nominal dose that leaves the device) and the respirable fraction (as percentage of the nominal dose (RFN) and as percentage of the emitted dose (RFE) of BDP that reaches the L compartment) were evaluated.

The same methodology was used for the TI determinations of the commercial product.

1. Emitted fraction:

$$\frac{\text{Emitted dose of BDP (compart. U + M + L)} \times 100}{\text{Nominal dose}}$$

Table 1
Identification and percentage of the emitted fraction, RFN and RFE of the formulations 1Lac, 2Lac, 1Tre, 2Tre, 1Res, 2Res and commercial product (mean ± SD, $n = 3$).

	Components of the physical mixture		Formulation nomination	Emitted fraction	RFN	RFE
	(BDP:γ-CYD)	Carrier				
Lactose	(2:5)	93	1Lac	75.3 ± 5.25	35.9 ± 4.53	47.6 ± 5.18
	(2:5)	193	2Lac	71.7 ± 2.22	35.2 ± 2.80	49.1 ± 3.56
Trehalose	(2:5)	93	1Tre	81.7 ± 2.95	36.6 ± 2.11	44.8 ± 2.47
	(2:5)	193	2Tre	81.9 ± 3.21	34.2 ± 1.19	41.7 ± 0.657
Respitose	(2:5)	93	1Res	58.3 ± 3.43	23.5 ± 1.05	40.4 ± 2.62
	(2:5)	193	2Res	72.4 ± 2.18	26.6 ± 1.07	36.8 ± 1.10
Commercial product				78.6 ± 8.45	12.6 ± 1.78	16.1 ± 2.46

2. Respirable fraction (percentage of the nominal dose):

$$\frac{\text{Dose of BDP into compart. L} \times 100}{\text{Nominal dose}}$$

The data were statistically analysed by calculating the mean, standard deviation (SD) and relative standard deviation (RSD). The differences between the means were compared by analysis of variance (ANOVA) using *F*-test. For significant *F*, Student's *t*-test was applied, to evaluate the pairs with statistically different means. In all the statistical analyses, a 5% level of significance was considered.

2.2.6. Determination of the moisture content

The residual moisture content of the powders (for each formulation $n = 3$) was determined via loss-on-drying using a Mettler LP16 moisture analyser, including an infrared dryer and a Mettler PM460 balance (Mettler-Toledo, Zaventem, Belgium). A sample of 0.5 g was dried at 105 °C for 5 min.

3. Results and discussion

3.1. Particle-size distribution

The BDP in the micronised form as starting material, prior to spray-drying, presents a unimodal particle distribution and very regular shape (Fig. 1A), with an appropriate size for DPI formulations (95% of particles below 1.8 μm). Whilst for γ-CYD raw material, it is possible to observe (Fig. 1B) the presence of two particle populations, with a size distribution profile and a dimension that are not appropriate for pulmonary administration (40% of particles below 5.95 μm and 95% of particles below 37.2 μm).

Fig. 2 demonstrates that, through the spray-drying, it was possible to obtain a unimodal size distribution within a size range appropriate for pulmonary deposition (95% of particles below 3.71 of μm).

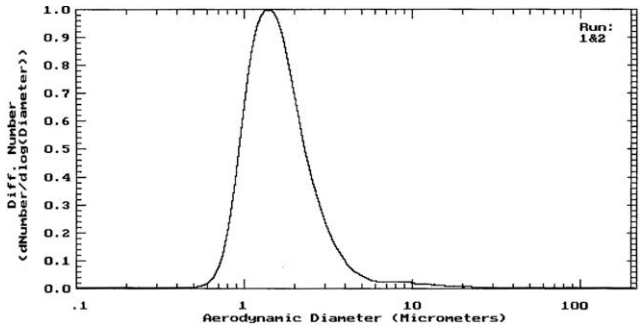

Fig. 2. Particle-size distribution, by number of particles, of the complex BDP:γ-CYD obtained by spray-drying.

Fig. 3A and B shows that through the sieving process, lactose and trehalose, besides the intended fraction (63–90 μm), present also a high percentage of fine particles (lactose: 45% < 4.50 μm; trehalose: 45% < 4.65 μm), which could impact aerosolisation [25,26].

Respitose presents a different particle-size distribution profile, i.e., only one particle population (Fig. 3C) being interesting to evaluate when compared with the bimodal distributions of lactose and trehalose.

In Fig. 4, the BDP and γ-CYD morphologies before any processing are observed. BDP particles have irregular and elongated shape caused by the micronisation process used during its production, while γ-CYD particles present large size and cubical and prismatic shapes.

The spray-drying process resulted in a BDP:γ-CYD complex with appropriate size distribution and morphology (more spherical particles) for inhalation delivery (Fig. 5). The influence of the conditions of spray-drying process in the morphology of particles is discussed later.

3.2. Aerosol performance

The emitted dose values obtained with all formulations are characteristic of the Rotahaler device [27].

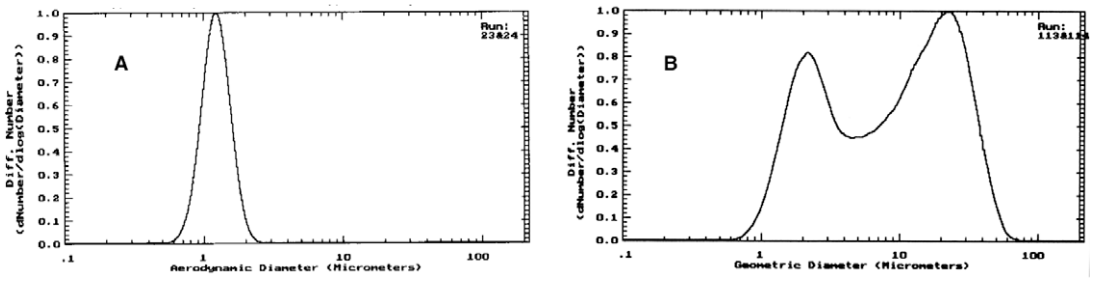

Fig. 1. Particle-size distribution, by number of particles: (A) BDP and (B) γ-CYD.

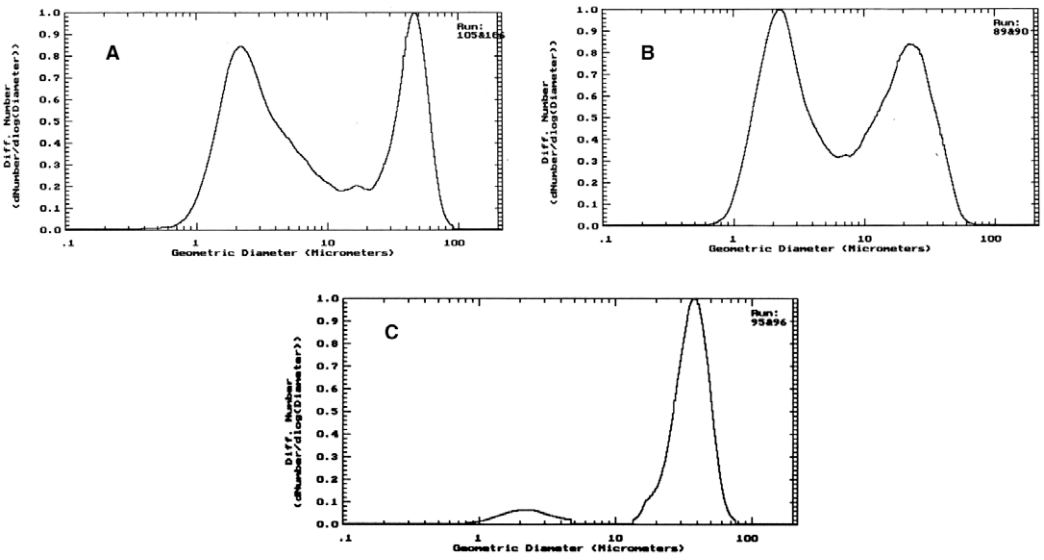

Fig. 3. Particle-size distribution, by number of particles, of the carriers (A) Lactose, (B) Trehalose and (C) Respitose.

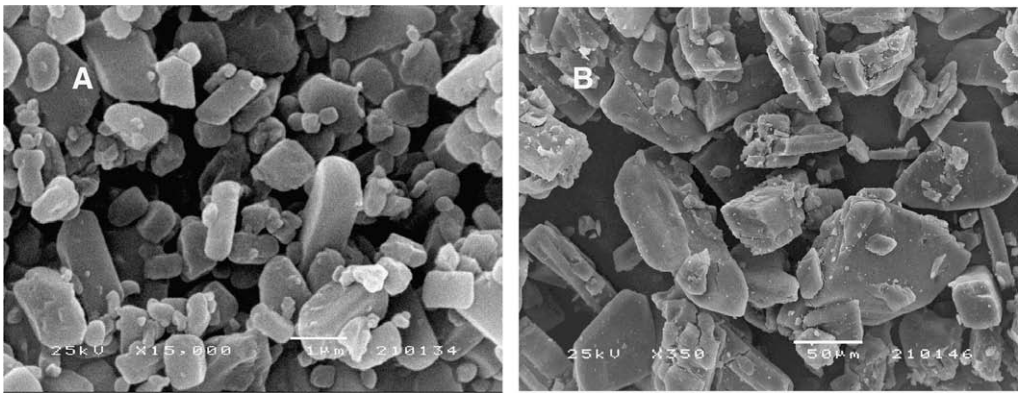

Fig. 4. SEM characterisation of the (A) BDP and (B) γ-CYD.

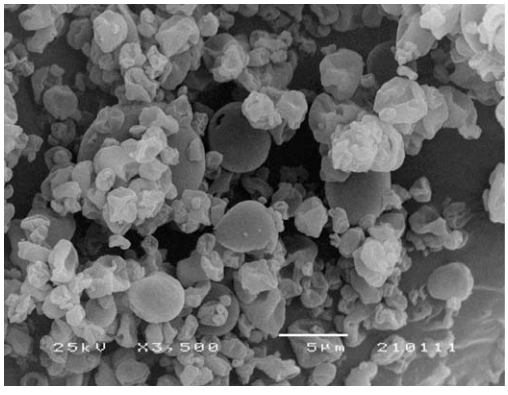

Fig. 5. SEM characterisation of the BDP:γ-CYD complex obtained by spray-drying.

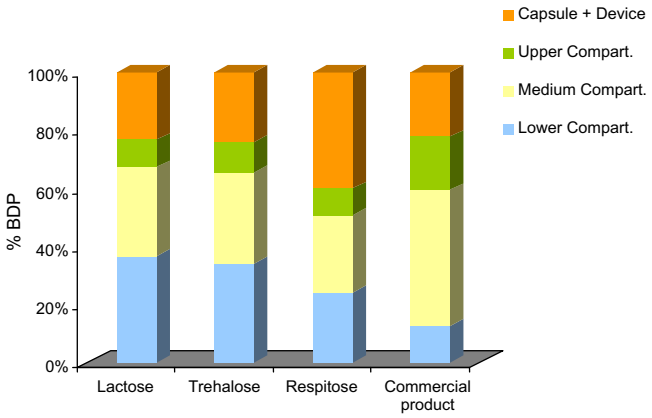

Fig. 6. BDP distribution in the TI compartments for formulations (2:5):93 and commercial product (percentage of the nominal dose).

In relation to the influence of the carrier on the delivery of BDP, the following conclusions can be drawn from analysis of the results shown in Figs. 6 and 7 and Table 1:

1. The lactose and trehalose formulations presented similar RFN in formulation 1 as in formulation 2. However, the formulation 2Tre demonstrates less-efficient dispersion, evidenced by the statistically lower RFE values than those of the formulation 2Lac, which could result from the physical (particle size) and/or chemical (adhesive interaction force) properties of the carrier systems.
2. The formulations with respitose as carrier showed poorer inhalation performance, demonstrating that the performance of this carrier is not as efficient as the performance of the mixture of

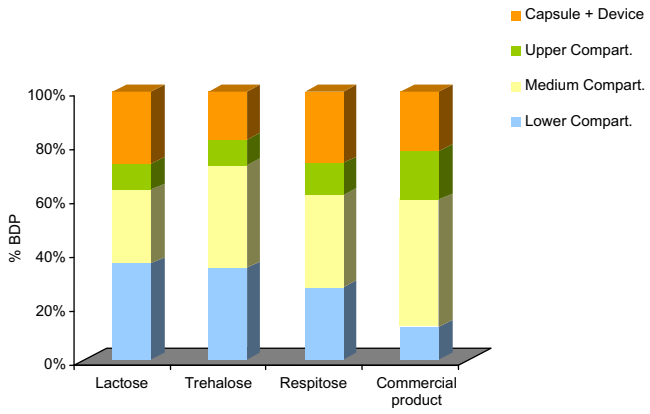

Fig. 7. BDP distribution in the TI compartments for formulations (2:5):193 and commercial product (percentage of the nominal dose).

different particle powder sizes, such as in the lactose or trehalose. These results confirm that the presence of fine carrier particles is advantageous; therefore, the links between these particles and the BDP fine particles are more easily broken, during the inhalation process, than those formed with carrier particles of larger dimensions [28–30].

3. All the prepared formulations showed some difficulty in the release of the powder from the capsule (Figs. 6 and 7), maybe due to the high moisture content flow (Table 2) of the formulations and, consequently, because of their adhesive properties and difficulties of entrainment into the air flow (maximum emitted fraction: 81.9% ± 3.2 in the formulation 2Tre; minimum emitted fraction: 58.3% ± 3.4 in the formulation 1Res).
4. In all prepared formulations, the deposition of BDP in the upper compartment was lower than that of the commercial product (Figs. 6 and 7). This result is important because it could potentially translate to a reduced deposition in the oropharynx *in vivo*, since oropharyngeal deposition of corticosteroids is associated with oral candidiasis.
5. In all the prepared formulations, the BDP content in the compartment representing the alveolar zone was statistically superior to that of the commercial product. This result is more relevant in the case of the formulation 1Res, which presented the lowest value of emitted fraction (58.3% ± 3.4), and lower than that of the commercial product (78.6% ± 8.5). Thus, it allowed the attainment of a superior RFN related to that of the commercial product, showing the best aerodynamic properties of this formulation.
6. Whilst differences in RFN are noted among the prepared formulations, all may deliver a sufficient dose of BDP to the lungs (maximum: 36.6% ± 2.1 for formulation 1Tre; minimum: 23.5% ± 1.1 for formulation 1Res).
7. It can thus be concluded that the particle size of the carrier and the processing of the BDP through spray-drying are important determinants of aerosolisation performance of BDP.

Li et al. [20] also concluded that DM-β-CYD showed considerable potential as a dispersibility enhancer to increase the fine particle fraction and functional deposition of non-viral gene therapy dry powder dispersion.

Table 2
Moisture content for the formulations (2:5):93 and (2:5):193.

Formulation	Moisture content
1Lac	6.03
1Tre	5.71
1Res	7.50
2Lac	6.32
2Tre	5.71
2Res	6.72

3.3. The influence of the carrier ratio

Studies performed with several carriers (lactose, trehalose and respitose) have shown that trehalose did not present advantages over lactose. Lactose was used since it is the mostly used carrier in inhalation formulations. Lactose is also a cheaper carrier and more accessible in the market.

On the other hand, fine particles exist in this carrier with potential to reach the alveolar zones; the lesser its ratio in the final physical mixture, the less probable will be the reaction that can result from its deposition at the respiratory tract. Therefore, between the formulations 1Lac and 2Lac, formulation 1Lac was selected (Table 1). Even if fine particles of lactose are deposited in the lung, as it is soluble, they will be easily cleared, and furthermore, lactose has a well-established safety profile for inhalation [32,33].

Concerning the carrier lactose, for formulation 1Lac *vs.* formulation 2Lac, statistics analysis (Student's *t*-test) has not shown significant differences (at a confidence level of 95%) for the parameters that characterise the aerodynamic properties of the powders for inhalation (emitted fraction, RFN and RFE).

Concerning the carrier trehalose, for formulation 1Tre *vs.* formulation 2Tre the formulation 1Tre also presented similar inhalation performances to those of the formulation 2Tre, with no statistically significant differences (at a confidence level of the 95%), between the mean values of the analysed parameters (emitted fraction, RFN and RFE)

Concerning the carrier respitose, for formulation 1Res *vs.* formulation 2Res, the statistical analysis (Student's *t*-test) demonstrated that the formulation 2Res presents a higher emitted fraction and RFN than the formulation 1Res. The RFE of the formulation 2Res is also statistically superior. It is not possible to identify whether the improvements seen in this formulation result from the slight reduction in the moisture content or from the increase in the carrier ratio of the physical mixture, or from the association of both.

The parameter which best describes the potential for deposition in the lung is the RFN, and for this parameter, it is possible to rank the formulations thus:

$$1Lac = 2Lac = 1Tre > 2Tre > 2Res > 1Res > \text{commercial product}.$$

All products obtained by spray-drying (BDP:γ-CYD complexes) were mixed with lactose in a mass ratio of 7:93 (complex:carrier). For clarity of discussion, spray-dried powders obtained by different experimental conditions will be identified as SD and their mixtures with lactose as MF (descriptors reported in Table 3).

Although the fine particles of complex BDP:γ-CYD, achieved under several experimental conditions, have different morphologic characteristics and different particle-size distributions, the retention percentage in the delivery device was similar (about 25%), which is consistent with previous literature on Rotahaler performance [27]. These results confirm that the moisture (Table 2) and, possibly, the inhalation device are the main responsible factors for this limitation in the prepared formulations.

3.4. Study of spray-drying process conditions

Careful selection of operating parameters can play a significant role in obtaining high quality product during spray-drying [7].

From all parameters of the spray-drying process, the T_{out} (which is indicator of the drying speed of the atomised droplets), is the parameter that has greater influence on the characteristics of the

Table 3
Identification and yield of the BDP:γ-CYD (2:5) complex obtained by spray-drying (SD) in different experimental conditions, and identification of the resulting physical mixture (MF) with lactose – (2:5):93.

Solution flow (ml/min)	Air inlet temperature (°C)	BDP:γ-CYD complex identification	Spray-drying process yield (%)	Resulting formulation identification
5	55	SD:5–55	52.8	MF:5–55
5	70	SD:5–70	51.0	MF:5–70
8	55	SD:8–55	44.4	MF:8–55
8	70	SD:8–70	42.7	MF:8–70
11	55	SD:11–55	34.2	MF:11–55
11	70	SD:11–70	36.2	MF:11–70

resultant product, such as the shape, surface morphology of the particles and moisture content [11]. Also, the drying time for droplets depends on process conditions such as flow rate, pump rate, aspiration rate and heat [7].

In our experiment, T_{out} is a dependent variable, being influenced by T_{in} and the solution flow [34]. Therefore, in the present work, the influence of T_{out} and these variables on the aerodynamic characteristics of the powder obtained was evaluated.

The maximum T_{in} was set to 70 °C, because at higher temperatures, the evaporation of the hydro-alcoholic solvent takes place in the atomisation cone with the consequent powder deposition at this level.

The lowest value of T_{in}, 55 °C, corresponds to the minimum value that allows the use of a solution feeding flow of 11 ml/min without condensation in the collector.

Then powders were prepared by spray-drying at two T_{in}, 55 °C and 70 °C, and three feeding solution flows: 5, 8 and 11 ml/min.

This study aimed at evaluating (a) the influence of these variables on the properties of the spray-drying powder and (b) the effect of these different properties on the inhalation performance of the formulation of BDP for pulmonary delivery.

3.5. Characterisation of the experimental conditions

It is possible to establish a relationship between the experimental conditions used and T_{out} which varies in direct proportion with T_{in} and shows an inverse relationship with the feed solution flow rate. These results are in agreement with previous publications on spray-dried protein powders [34,35]. The result of the determination of the yield for each one of the experimental conditions is shown in Table 3. The yield obtained from spray-drying constitutes a limitation to the use of this technique. The typical yield from a spray-dryer is between 20% and 50% [7]. However, Buchi has introduced a high-performance cyclone to improve the yield up to 70%.

The results seem to indicate that the yield of the spray-drying process is influenced by the flow of feeding solution, as yields were higher for the lowest flow (5 ml/min). However, no relation with the T_{in} was noticed.

The low yields achieved with the flows of 8 and 5 ml/min are characteristic of this methodology and in the range 40–70%, described in literature [35,36]. The yields of 34.2% and 36.2%, achieved with the flow of 11 ml/min (Table 3), are very low, and these experimental conditions should not be considered as the formulations prepared with this powder have poor aerodynamic characteristics. These low values could be justified by the biggest number of atomised particles that in proportion adheres more to the walls of the drying chamber, its removal for the cyclone collector being more difficult, leading to lower yield values in the process.

According to Broadhead et al. [35], the difficulties found at the laboratory level, considering the spray-drying process, are solved during the scaling-up process, as at the industrial level, the obtained yields are superior.

The following comments are highlighted:

- The particle-size distribution curve of the powder obtained in conditions SD:5–70 presents a similar profile to a normal distribution curve;
- The sample SD:11–55 presents bigger particles, where 95% < 7.52 μm, but 90% have diameter < 5.51 μm;
- In samples SD:11–55 and SD:11–70, it is possible to identify the slight appearance of a second particle population;
- For equal values of T_{in}, seems that lower flows originate more homogeneous populations (lower SD values). This could be justified by the fact that with lower flows, less particles coexist drying simultaneously and less agglomerations occur, generating more homogeneous powders;
- For the same flow, the temperature of 70 °C originates more homogeneous particles (lower SD values). These results estimate, like in the previous point, that the faster is the drying process, lower tendency for some particles to agglomerate and originate populations of bigger dimensions.

In summary, in a general way, it was verified that the most homogeneous populations were obtained with the higher T_{out} values, which correspond to the lowest feeding flow values and higher T_{in} values.

The initial morphologic characterisation of the obtained products in some conditions of spray-drying gives some indication on its aerodynamic characteristics. Thus, SEM images of the powders are obtained by spray-drying under several conditions: (A) SD:5–55; (B) SD:5–70; (C) SD:8–55; (D) SD:8–70; (E) SD:11–55; (F) SD:11–70 are shown in Fig. 8.

In general, particles present smooth surfaces, but according to different experimental conditions, they present different shapes.

Concerning SD:8–55, SD:11–55 and SD:11–70, few spherical particles are observed, the presence of irregular particles being evident, some with donut shape, other more oval- or concave-shaped and many collapsed "raisin-like" particles.

The shifts from sphericity can lead to an increase in the interactions between particles due to a bigger surface contact, causing more difficulties on flowing and redispersion of the powder [34].

When a carrier is added, the fine particles surface increases; this may lead to the formation of very strong links between these two entities. This may cause difficulties in breaking the links in the inhalation process.

In samples SD:5–55, SD:5–70 and SD:8–70 (specially in the last two), it is possible to identify some particles completely spherical, with smooth surfaces. These characteristics indicate a great potential to reach the alveolar zones after inhalation. The particles that are not spherical present more regular and homogeneous shapes and more close to spheroid shape than the samples previously referred.

The images obtained by SEM seem to indicate that high flows are not so favourable to get spherical particles.

Thus, considering the particles morphology, a particle-size distribution approached to the normal one, the powder SD:5–70 seems to be most promising in aerodynamic terms.

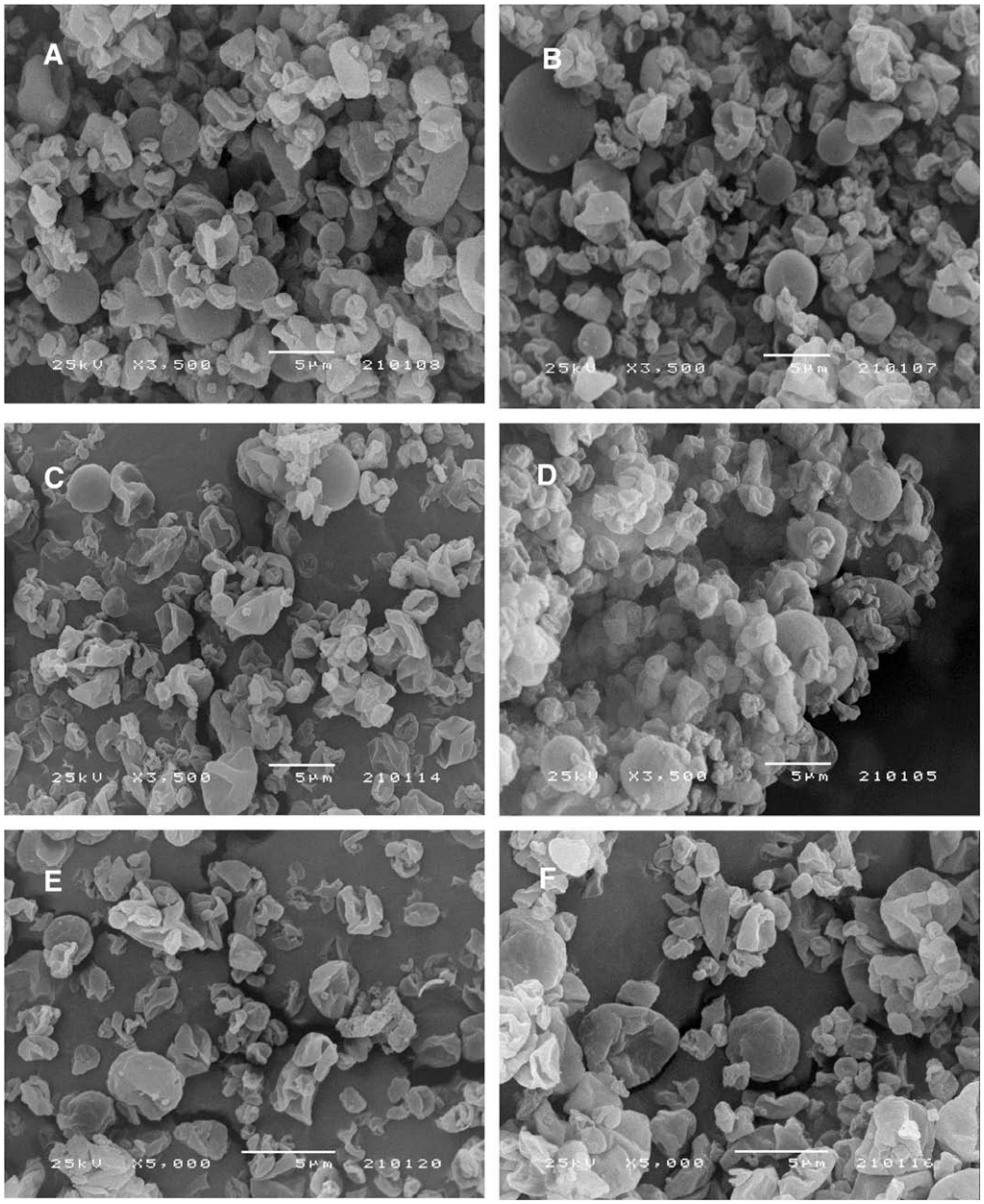

Fig. 8. SEM images of the powders obtained by spray-drying under several conditions: (A) SD:5–55; (B) SD:5–70; (C) SD:8–55; (D) SD:8–70; (E) SD:11–55; (F) SD:11–70.

The influence of T_{out} on the moisture content of the final product was also studied (Fig. 9). Broadhead et al. [35] obtained similar results concerning the influence of T_{out} on the moisture. There are other studies, where no changes on this parameter were observed with different experimental conditions [34].

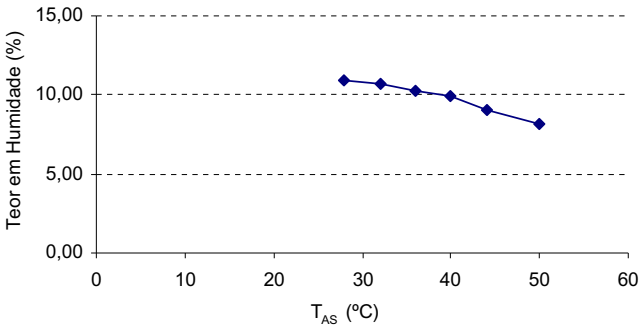

Fig. 9. Variation of the moisture content as function of T_{out}.

It is evidenced on this studied system that is possible to minimize the moisture content of the obtained product if working with the highest T_{out} values.

3.6. Carrier

Similar mean values of BDP retention in the TI upper compartment were assayed for all formulations (minimum: 10.2% ± 0.6 in the MF:5–70; maximum: 12.7% ± 0.8 in the MF:8–55), and the lowest among all compartments.

It is also verified that in the MF:5–55, in the MF:5–70 and in the MF:8–70, the lower compartment held greater BDP percentage; this is a good indicator of the potential of these conditions of spray-drying for the particle preparation with adequate characteristics to the pulmonary deposition. In the other formulations, although the biggest amount of BDP was assayed in the medium

compartment, the minimum value obtained in the lower compartment was of 25.6% ± 0.1.

In all the formulations, the maximum assayed value in the representative compartment of the lower respiratory tract was 38.4% ± 0.5 for formulation MF:5–70.

One evidences, also, that in the two formulations where the complex was prepared with a feeding flow of 11 ml/min, the percentage of BDP assayed in the lower compartment was very low (RFN: 25.6% ± 0.1 in the MF:11–55 and 27.4% ± 1.5 in the MF:11–70) (Fig. 10).

It could be hypothesised that the morphology of particles produced at high solution flow rate confers a greater degree of interaction with the carrier which is then detrimental to efficient aerosolisation.

The MF:8–55 showed slightly superior RFN values compared with MF:11–55 and MF:11–70. The application of Student's *t*-test ($\alpha = 0.05$) demonstrates, however, no statistically significant differences, to a reliable level of 95%, between the mean value of MF:8–55 and of the MF:11–70 ($p = 0.222$).

The best RFN values were obtained for MF:5–55: 34.9% ± 1.9; MF:8–70: 36.6% ± 0.6 and MF:5–70: 38.4% ± 0.5. A statistical comparison between the mean values of these formulations, by the application of a Student's *t*-test ($\alpha = 0.05$), demonstrated that no significant statistical differences were found between formulations MF:8–70 and MF:5–55 ($p = 0.142$), but the RFN values of the MF:5–70 are statistically superior to those of the MF:8–70 ($p = 0.00736$). This result allows to predict that formulation MF:5–70 is the one that delivers greater amount of BDP to the lungs.

Also in the RFE, the best results were obtained for these three formulations: MF:5–55: 47.1% ± 1.9; MF:8–70: 49.3% ± 0.8 and MF:5–70: 51.2% ± 0.5. It is extremely interesting to verify the direct relation of T_{out} in the RFE (Fig. 11).

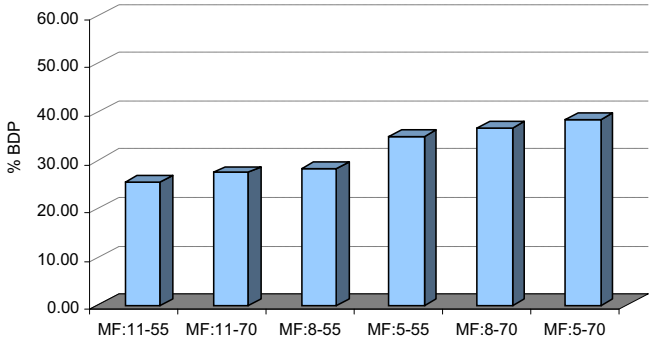

Fig. 10. RFN of the formulations prepared with the spray-dried powders in different experimental conditions.

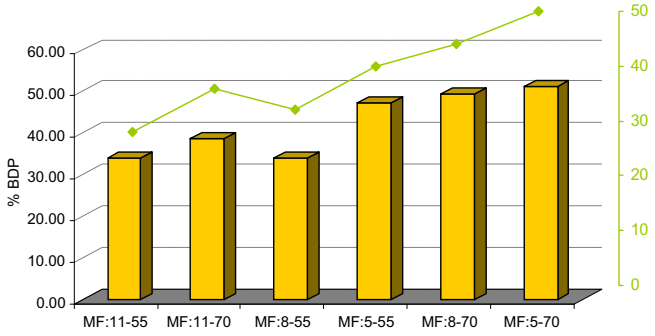

Fig. 11. RFE of the formulations prepared with the spray-dried powders in different experimental conditions and its relation with T_{out}.

The results confirm that different experimental conditions in the spray-drying process, especially the T_{out}, confer different inhalation characteristic to the particles and change the RFN from 25.6% to 38.4% and the RFE from 33.8% to 51.2%.

Concerning the RFE (the parameter that characterises the formulations in relation to the fine particles characteristics and its capacity of dispersion into the carrier), it is verified that the results are in agreement with the prediction of the particles morphology analysis and the particle-size distribution profile. In fact, the samples with more spherical and regular particles and narrower particle-size distributions, MF:5–70, MF:8–70 and MF:5–55 were those that demonstrated better aerodynamic performances. For the samples 8:55, 11:55 and 11:70, the presence of some particles with dimensions superior to 3 μm and perhaps aggregation, favoured by the most irregular surface of the particles, may have resulted in the formation of aggregates with the dimensions superior to 7 μm, which are not able to reach the lower compartment of TI, resulting in lower RFN and RFE.

3.7. Influence of the air inlet temperature (T_{in})

To evaluate the influence of T_{in} on the inhalation performance of the final formulations, their RFN and RFE will be compared.

The results evidence that the highest RFN values are always achieved with T_{in} of 70 °C and are statistically superior to the results obtained with T_{in} of 55 °C (Table 4). The only exception occurred with the flow of 11 ml/min, where the RFN mean values obtained with the MF:11–70, although numerically superior, are statistically similar to those of the MF:11–55.

Statistical analysis has shown that, for all the flows tested, the values for RFE at 55 °C are statistically inferior to those obtained at 70 °C, proving that the temperature of 70 °C allows to obtain powders for pulmonary administration with better aerodynamic characteristics (Table 4).

The results of the statistical analysis demonstrate that, to a reliable level of 95%, the differences between the mean values of RFN and RFE are statistically significant, the use of feeding flows of 5 ml/min being more advantageous, with the two T_{in} values. The only exception is that RFE values of the MF:8–55 are statistically similar to those of the MF:11–55.

It is verified, therefore that, for 55 °C, the reduction in the flow from 11 ml/min to 8 ml/min does not improve the characteristics of fine particles. This may be related with the very low T_{out} values in these experimental conditions.

In conclusion, the *in vitro* tests carried out demonstrate that in the spray-drying process, the deposition of BDP at the level of the pulmonary ramifications is favoured by: (a) superior air inlet temperatures and (b) reduced feeding solution flow rates. The flow of 5 ml/min associated to a T_{in} of 70 °C has shown to be more favourable in the processing of BDP, in the form of complex with γ-CYD.

Table 4
Student's *t*-test: variation of the RFN and the RFE as function of T_{out} – ($\alpha = 0.05$, 1-tail).

	Degrees of freedom	RFN		RFE	
		t value	*p* value	*t* value	*p* value
MF:5–55 – MF:5–70	4	3.02	0.0195*	3.46	0.0128*
MF:8–55 – MF:8–70	4	11.1	0.000186*	21.8	0.0000131*
MF:11–55 – MF:11–70	4	2.04	0.0887	3.30	0.0150*

* $p < 0.05$.

4. Conclusions

The spray-drying methodology has shown to be very suitable for BDP:γ-CYD complex formation and allowed a better uniformity of the mixture.

The particles mean diameter has little changes with the different experimental conditions, and these conditions have more influence on the morphology, particle-size distribution and moisture content of the final powders.

The results of the TI *in vitro* tests indicate that the preparation of particles with right characteristics for lung deposition, by spray-drying, is favoured by high T_{in} and low solution flows, which result in higher T_{out} values. T_{out} parameter has shown to influence RFE, in direct proportion by more rapid drying, giving smaller denser particles with smaller spread of size distribution.

Using a 5 ml/min flow, 70 °C T_{in} with consequent 50 °C T_{out}, particles with the best inhalation characteristics were obtained; the physical mixture with lactose in the 7:93 ratio presented the best values of RFN (38.4% ± 0.5) and RFE (51.2% ± 0.5). Using 11 ml/min flow, for all T_{in}, the low T_{out} values had lead to the preparation of powders with high moisture content. The particles obtained with this flow have irregular form and evidence of a bimodal particle-size distribution. These characteristics lead to very low values of RFN (25.6% ± 0.1 for MF:11–55 and 27.4% ± 1.5 for MF:11–70) and RFE (33.8% ± 0.7 for MF:11–55 and 38.6% ± 2.4 for MF:11–70).

The main limitation of the developed formulations was the percentage of BDP retained into the capsule and in the inhalation device, which could be related with the moisture content of the formulations. The optimisation of this parameter will be able to lead to a reduction of the agglomeration of particles and reduction of its adhesive properties, with consequent better dispersion of the formulation and attainment of higher emitted fraction values.

References

[1] C.L. Leach, Improved delivery of inhaled steroids to the large and small airways, Resp. Med. 92 (Suppl. A) (1998) 3–8.
[2] B.J. Lipworth, C.M. Jackson, Safety of inhaled and intranasal corticosteroids, Drug Safety 23 (1) (2000) 11–32.
[3] GINA Report, Global Strategy for Asthma Management and Prevention, 2007. <http://www.ginasthma.com/> (accessed 19.02.08).
[4] H.M. Cabral Marques, J. Hadgraft, I.W. Kellaway, G. Taylor, Studies of cyclodextrin inclusion complexes. Part IV. The pulmonary absorption of salbutamol from a complex with HP-β-cyclodextrin in rabbits, Int. J. Pharm. 77 (1991) 303–307.
[5] R.A. Rajewski, V.J. Stella, Pharmaceutical applications of cyclodextrins. 2. *In Vivo* drug delivery, J. Pharm. Sci. 85 (11) (1996) 1143–1169.
[6] C. Vozone, H.M. Cabral Marques, Complexation of budesonide in cyclodextrins and particle aerodynamic characterization of its solid form for dry powder inhalation, J. Incl. Phenom. Macrocycl. Chem. 44 (2002) 111–115.
[7] S.A. Shoyele, S. Cawthorne, Particle engineering techniques for inhales biopharmaceuticals, Adv. Drug Deliv. Rev. 58 (2006) 1009–1029.
[8] Y.F. Maa, P.A. Nguyen, T. Sweeney, S.J. Shire, C.C. Hsu, Protein inhalation powders: spray-drying *vs.* spray freeze drying, Pharm. Res. 16 (2) (1999) 249–255.
[9] S.T. Tzannis, S.J. Prestrelsky, Activity-stability considerations of trypsinogen during *spray-drying*: effects of sucrose, J. Pharm. Sci. 88 (3) (1999) 351–359.
[10] S.T. Tzannis, S.J. Prestrelsky, Moisture effects on protein–excipient interactions in spray-dried powders. Nature of destabilizing effects of sucrose, J. Pharm. Sci. 88 (3) (1999) 360–369.
[11] Y.F. Maa, P.A. Nguyen, J.D. Andya, N. Dasovish, T.D. Sweeney, S.J. Shire, C.C. Hsu, Effect of *spray-drying* and subsequent processing conditions on residual moisture content and physical/biochemical stability of protein inhalation powders, Pharm. Res. 15 (5) (1998) 768–775.
[12] J.D. Andya, Y.F. Maa, H.R. Costantino, P.A. Nguyen, N. Dasovish, T.D. Sweeney, C.C. Hsu, S.J. Shire, The effect of formulation excipients on protein stability and aerosol performance of spray-dried powders of a recombinant humanized anti-IgE monoclonal antibody, Pharm. Res. 16 (3) (1999) 350–358.
[13] T.P. Foster, M.W. Leatherman, Powder characteristics of proteins spray-dried from different spray-dyers, Drug Dev. Ind. Pharm. 21 (15) (1995) 1705–1723.
[14] R. Almeida, H. Cabral Marques, Pulmonary administration of beclomethasone: ã-cyclodextrin complex, in: D. Duchêne (Ed.), Proceedings of the 12th International Cyclodextrin Symposium, de Santé ed., APGI Publishing, Montpellier, França, 2004, pp. 889–892.
[15] J.M.C. Leite Pinto, H.M. Cabral Marques, Beclomethasone – cyclodextrin inclusion complex for dry powder inhalation, STP-Pharma 9 (3) (1999) 253–256 (May–June special issue in honour of Prof. Szejtli).
[16] K. Fromming, J. Szejtli, Pharmacokinetics and toxicology of cyclodextrins. Cyclodextrins in Pharmacy, vol. 5, Kluwer Academic Publishers, 1994, pp. 33–44 (Chapter 3).
[17] H.M. Cabral Marques, Structure and properties of cyclodextrins. Inclusion complex formation, Rev. Port. Farm. XLIV (2) (1994) 77–84.
[18] T. Nakate, H. Yoshida, A. Ohike, Y. Tokunaga, R. Ibuki, Y. Kawashima, Improvement of pulmonary absorption of cyclopeptide FK224 in rats by co-formulating with β-cyclodextrin, Eur. J. Pharm. Biopharm. 55 (2003) 147–154.
[19] A. Hussain, J. Arnold, M. Khan, F. Ahsan, Absorption enhancers in pulmonary protein delivery, J. Control. Release 94 (2004) 15–24.
[20] H.-Y. Li, P.C. Seville, I.J. Williamson, J.C. Birchall, The use of absorption enhancers to enhance the dispersibility of spray-dried powders for pulmonary gene therapy, J. Gene Med. 7 (2005) 1035–1043.
[21] T. Kinnarinen, P. Jarho, K. Järvinen, T. Järvinen, Pulmonary deposition of a budesonide/gamma-cyclodextrin complex in vitro, J. Control. Release 90 (2) (2003) 197–205.
[22] B. Evrard, P. Bertholet, M. Gueders, M.-P. Flament, G. Piel, L. Delattre, A. Gayot, P. Leterme, J.-M. Foidart, D. Cataldo, Cyclodextrins as potential carrier in drug nebulization, J. Control. Release 96 (2004) 403–410.
[23] European Pharmacopoeia, fifth ed., Council of Europe, Strasbourg, France, 2005 (2.9.18. Preparation for inhalation: aerodynamic assessment of fine particles).
[24] H.M. Cabral Marques, P.C. Schmidt, Comparison of the *in vitro* deposition of dry powder inhalations by laser diffractometry and inertial impaction, Die Pharm. 57 (2002) 546–551.
[25] X.M. Zeng, K.H. Pandhal, G.P. Martin, The influence of lactose carrier on the content homogeneity and dispersibility of beclomethasone dipropionate for dry powder aerosols, Int. J. Pharm. 197 (2000) 41–52.
[26] S.K. Tee, C. Marriott, X.M. Zeng, G.P. Martin, The use of different sugars as fine and coarse carriers for aerosolised salbutamol sulphate, Int. J. Pharm. 208 (2000) 111–123.
[27] M. Hindle, P.R. Byron, Dose emissions from marketed dry powder inhalers, Int. J. Pharm. 116 (1995) 169–177.
[28] P. Lucas, K. Anderson, J.N. Staniforth, Protein deposition from dry powder inhalers: fine multiplets as performance modifiers, Pharm. Res. 15 (4) (1998) 562–569.
[29] P. Begat, D.A. Morton, J.N. Staniforth, R. Price, The cohesive–adhesive balances in dry powder inhaler formulations I: direct quantification by atomic force microscopy, Pharm. Res. 21 (9) (2004) 1591–1597.
[30] P. Begat, D.A. Morton, J.N. Staniforth, R. Price, The cohesive–adhesive balances in dry powder inhaler formulations II: influence on fine particle delivery characteristics, Pharm. Res. 21 (10) (2004) 1826–1833.
[32] P. Baldrick, D.G. Bamford, A toxicological review of lactose to support clinical administration by inhalation, Food Chem. Toxicol. 35 (7) (1997) 719–733.
[33] M. Karhu, J. Kuikka, T. Kauppinen, K. Bergström, M. Vidgren, Pulmonary deposition of lactose carriers used in inhalation powders, Int. J. Pharm. 196 (1) (2000) 95–103.
[34] Y.F. Maa, H. Costantino, P.A. Nguyen, C.H. Hsu, The effect of operating and formulation variables on the morphology of spray-dried protein particles, Pharm. Dev. Tech. 2 (3) (1997) 213–223.
[35] J. Broadhead, S.K.E. Rouan, I. Hau, C.T. Rhodes, The effect of process and formulation variables on the properties of spray-dried β-galactosidase, J. Pharm. Pharmacol. 46 (1994) 458–467.
[36] C.A. Dunbar, N.H. Concessio, A.J. Hickey, Evaluation of atomization performance in production of respirable spray-dried particles, Pharm. Dev. Tech. 3 (4) (1998) 433–441.