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Complexation of Budesonide in Cyclodextrins and Particle Aerodynamic Characterization of the Complex Solid Form for Dry Powder Inhalation

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Abstract

The purpose of this study is to evaluate the effect of budesonide-cyclodextrins (CDs) complex formation in the *in-vitro* aerodynamic properties of the dry powder produced for pulmonary delivery. Phase-solubility studies were performed using budesonide and β-CD, DM-β-CD and HP-β-CD. The complex budesonide:DM-β-CD revealed the highest stability constant (K_s = 3339.7 $\pm$ 4.76%; $n = 3$) and the solid powder was prepared by spray-drying. Complexation was evidenced by Differential Scanning Calorimetry (DSC). A physical mixture of budesonide and DM-β-CD was prepared for use as reference. The fine particle fraction and particle size distribution of both powders were assessed using Twin Stage Liquid Impinger (TSLI) and Aerosizer®LD, respectively. The content uniformity of the capsules filled (sd); (n) was 191.8 ($\pm$ 2.74) μg; (10) for the budesonide:DM-β-CD solid complex and 204.9 ($\pm$ 9.35) μg; (10) for the physical mixture. The emitted dose (rsd); (n) was 68.0% ($\pm$ 26.1%); (5) of the nominal dose (solid complex) and 70.6% ($\pm$ 12.6%); (5) (physical mixture). The fine particle fraction was 67.7% ($\pm$ 18.9%); (5) of the emitted dose (solid complex) and 39.8% ($\pm$ 16.9%); (5) (physical mixture). While no statistically significant difference was observed between the emitted dose means of both the solid complex and physical mixture, a statistically significant higher fine particle fraction mean was obtained for the solid complex. The results suggest that using a spray-dried CD complex powder for pulmonary drug delivery may increase the drug's respirable fraction and consequently its therapeutic efficacy.

Introduction

The efficacy of a dry powder inhaler (DPI) is related to the extent of drugs' deposition in the lung, which in turn depends on the delivery device characteristics, on the drug formulation and on the patients' inspiratory flow [1, 2]. The major issue in pulmonary delivery of dry powder drugs is still its low efficiency. With the conventional formulation and devices, generally only 10 to 20% of the emitted dose is deposited in the alveolus [1, 3].

The use of CDs in pulmonary delivery has been sparingly referred in the literature. Some authors have studied the drug:CD complexation's effect in delaying the pulmonary absorption of drugs [4]. Recently, CDs have been investigated as trans-membrane absorption promoters of polar macromolecules, like peptides or oligo-nucleotides, through the alveolar membranes [5, 6]. The present work intends to study how CDs affect the aerodynamic properties of powders and improve their flowability. Similar studies have been published elsewhere [7, 8].

The aerodynamic properties of a powder, namely its morphologic pattern and particle size distribution determine the degree of drug deposition in the respiratory tract, thereby affecting its therapeutic outcome. Particle sizes above 5 μm impact in the upper airways and are unable to reach the lower respiratory tract, where the drug is absorbed, decreasing its efficacy [9]. Additionally, the surface roughness limits the cohesive forces established between the particles, which to a certain extent, improve the powder fluidization and the drug amount that reaches the respirable fraction [10]. The spray-drying process produces small, spherical and amorphous powder particles with a certain degree of roughness and within a narrow particle size range [11], improving powder fluidization.

Budesonide is indicated for asthma and exerts its therapeutic effects through a local and systemic action. The pulmonary absorption of budesonide avoids the hepatic effect and increases the bioavailability of the drug. It is therefore interesting to increase the respirable fraction of this drug in order to improve its local and systemic effect.

Experimental

Material and methods

Budesonide, micronized powder, offered by Sicor, Societá Italiana Corticosteroidi, Italy; betacyclodextrin (β-CD), dimethylbetacyclodextrin (DM-β-CD), hydroxypropylbetacyclodextrin (HP-β-CD), Wacker-Chemie GmbH, Germany; ethanol 97%, Merck, Germany; FlowCaps®,

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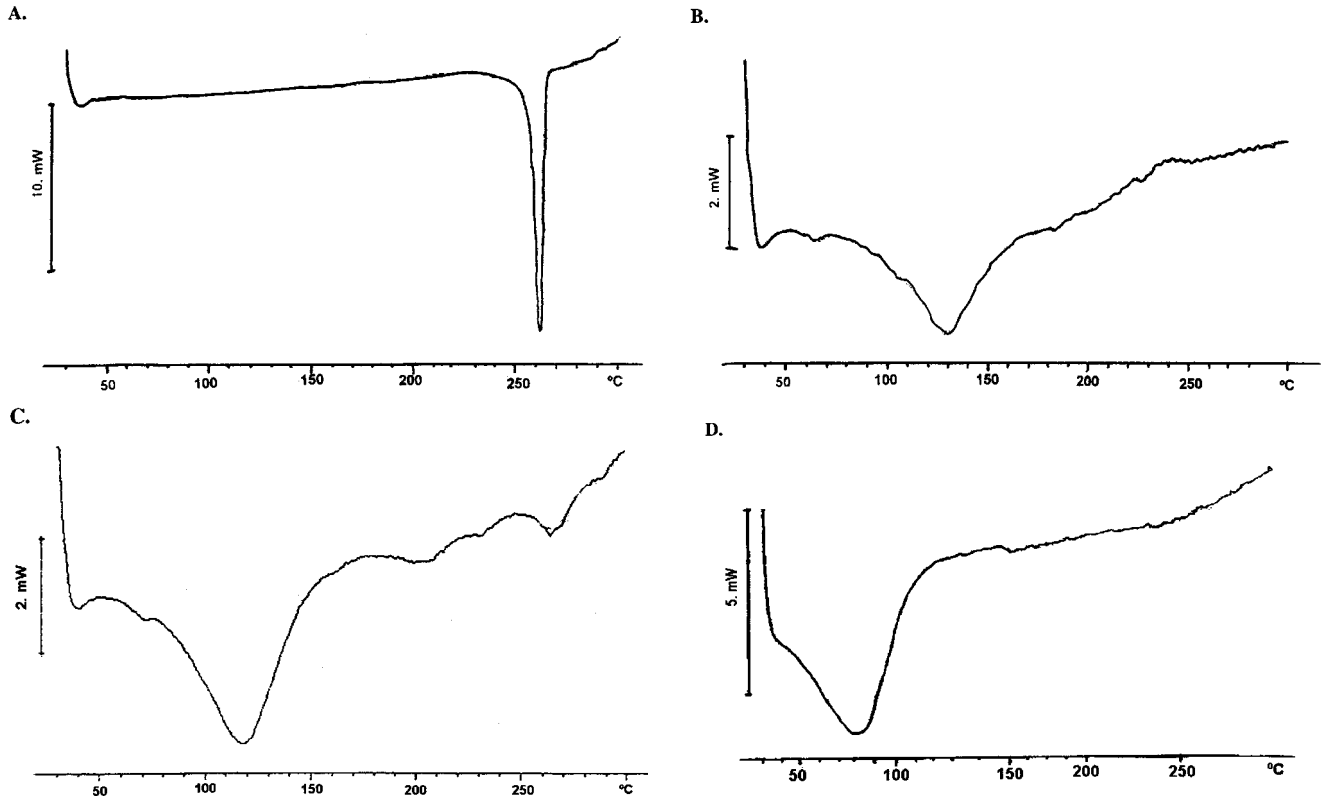

Figure 1. Endothermic curves obtained by DSC: (A) budesonide, (B) DM-β-CD, (C) budesonide:DM-β-CD physical mixture, (D) budesonide:DM-β-CD solid complex.

offered by Hovione FarmaCiencia SA, Portugal; hydroxypropyl-methyl cellulose (HPMC) capsules, number 4, Shionogi Qualicaps, Japan.

The complex formation stability constants of budesonide and the three CDs under study (β-CD, DM-β-CD and HP-β-CD) were determined using the phase-solubility techniques developed by Higuchi and Connors [12]. Stock saturated water solutions of each CD were prepared: β-CD, 0.012 mol L^{-1}; DM-β-CD, 0.20 mol L^{-1} and HP-β-CD, 0.20 mol L^{-1}, which were diluted to have five sampling points. About 50 mg of budesonide (greater above its solubility in water) was added to 10 ml of each solution prepared. The systems were brought to equilibrium (24 hours) by prolonged agitation (300 rpm), at 37 °C. Solutions were filtered by a 0.22 μm filter (Millex®-GS), diluted with ethanol 97% and budesonide's content was determined by spectrophotometry.

Phase-solubility diagrams were constructed for each CD by plotting the molar concentration of budesonide in solution on the vertical axes against the molar concentration of the CD on the horizontal axes.

The solid inclusion compound of budesonide and DM-β-CD in a 1:1 and 1:10 molar ratio, respectively, was prepared by spray-drying. A Mini Spray Dryer B-191, Büchi, Germany, was used in the following drying conditions: inlet temperature 120 °C; feed flow rate 5 ml min^{-1}; aspirator setting at 100% (35 m h^{-1}).

A physical mixture of budesonide and DM-β-CD, at a ratio 1:1 and 1:10, respectively, was prepared through sieving by 150 mesh and using a rotation mixer at 200 rpm during 4 minutes.

The device used was a capsule based passive dry-powder inhaler, FlowCaps®, developed by Hovione FarmaCiencia SA. Capsules were hand-filled with 6.3 mg of the solid complex and the physical mixture (1:10 ratio), equivalent to 200 μg of budesonide.

Physical measurements

Budesonide content was determined by spectrophotometry at 243 nm, Hitachi U2000. The samples were diluted in ethanol 97%.

Complex formation was evidenced by Differential Scanning Calorimetry (DSC), Mettler Toledo TA4000 System; TC11 TA processor.

The particle size distribution was determined in the solid complex and in the physical mixture powders by an Aerosizer®LD. The fine particle fraction in both powders was assessed by an impaction based apparatus – Apparatus A (Glass Impinger), Eur. Pharm., 4th edition, 2002, also known as Twin Stage Liquid Impinger (TSLI). The procedure followed Eur. Pharm. requirements, except for the air flow, which was adjusted to FlowCaps® air flow resistance: 30 $\pm$ 5 L min^{-1} during 5 s. The apparatus' components of the upper and lower stages and the capsules were washed separately and diluted to a volumetric flask of 100 ml

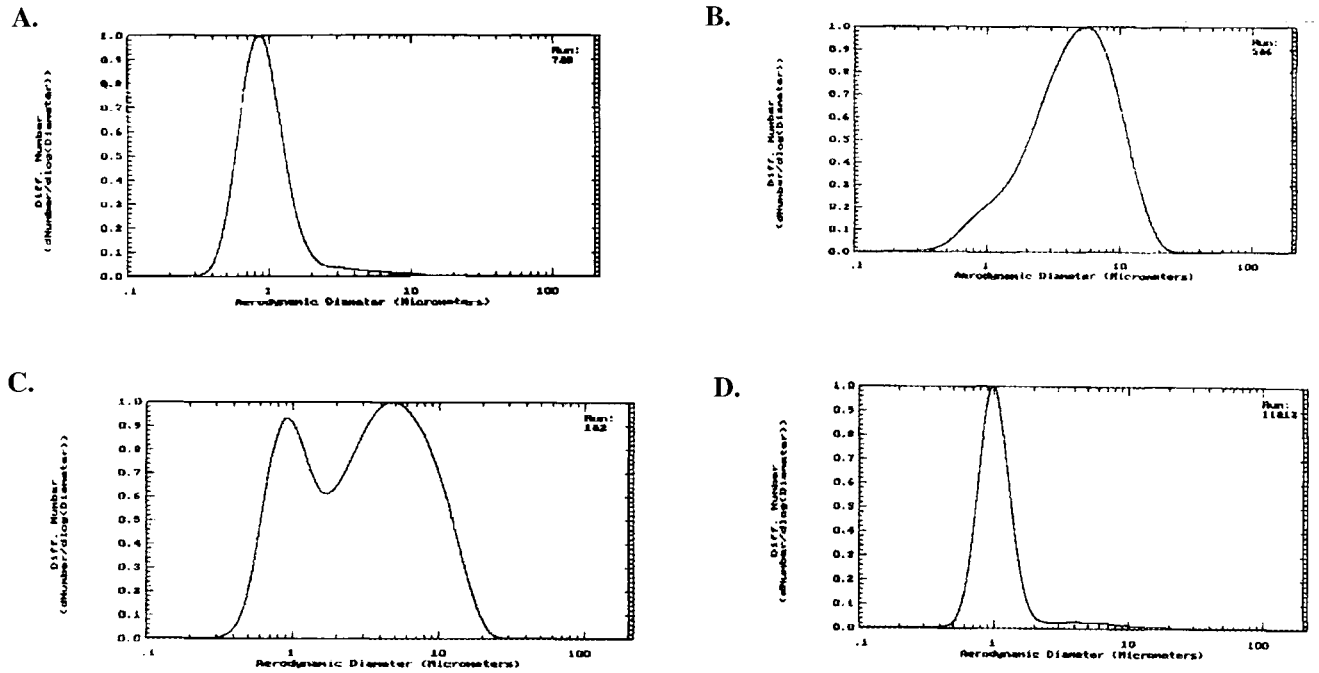

Figure 2. Particle size distribution by Aerosizer® LD, aerodynamic diameter in μm: (A) budesonide, (B) DM-β-CD, (C) budesonide:DM-β-CD physical mixture, (D) budesonide:DM-β-CD solid complex.

Table 1. TSLI emitted dose and capsule retained dose (average % in relation to nominal dose; rsd; $n = 5$) for budesonide:DM-β-CD 1:10 physical mixture and budesonide:DM-β-CD 1:10 solid complex)

Sample	Capsule retained dose (% of the nominal dose)		Emitted dose (% of the nominal dose)	
	Average	RSD %	Average	RSD %
Budesonide:DM-β-CD (1:10) Physical mixture	26.9%	29.9	70.6%	12.6
Budesonide:DM-β-CD (1:10) Solid complex	31.2%	61.6	68.0%	26.1

with ethanol 97%, after one discharge. The TSLI data were statistically treated using a comparison of means t-test.

Results and discussion

Determination of β-CD, DM-β-CD and HP-β-CD:budesonide complex stability constants

The extent of complex formation in an aqueous milieu is characterised by the stability constant (K_s) of the complex and depends on the affinity that the guest molecule has to the CD's cavity. The CD, the guest molecule and its complex equilibrium in solution is described by Equation (1).

$$K_s = [\text{complex}]/[\text{CD}][\text{guest}]. \quad (1)$$

The K_s for each CD included in the experiment and for budesonide were determined from the phase-solubility diagrams at equilibrium. K_s was calculated from the slope of the linear part of the curves and the interception point at the vertical axes (S_0) values according to Equation (2).

$$K_s = \text{slope} / S_0\,(1 - \text{slope}). \quad (2)$$

Preliminary studies revealed high stability constants for all CDs studied in the following order: DM-β-CD > HP-β-CD > β-CD. The reproducibility of the results was confirmed for DM-β-CD by repeating the phase-solubility analysis. The stability constant average, (rsd) of the three determinations were $K_s = 3339.7$ ($\pm$ 4.76%).

Evidence of complex formation by DSC

The inclusion of a guest molecule in a CD induces modification of various chemical and physical properties on the former [13]. The measurement of those changes is the basis of the methods used to confirm the complex formation. DSC evidences inclusion in a CD by the modification of the guest's molecule endothermic peak. The endothermic peak can be observed in the physical mixture, but is absent in the complex [13]. Figures 1A to 1D show the endothermic curve of budesonide, DM-β-CD and of budesonide:DM-β-CD physical mixture and solid complex, both at a 1:1 ratio.

Table 2. TSLI budesonide's deposition assay (average % in relation to the emitted dose; rsd; $n = 5$) for budesonide:DM-β-CD 1:10 physical mixture and budesonide:DM-β-CD 1:10 solid complex

Sample	Lower stage (% in relation to emitted dose)		Upper stage (% in relation to emitted dose)	
	Average	RSD %	Average	RSD %
Budesonide:DM-β-CD (1:10) Physical mixture	39.8%	16.9	60.2%	11.2
Budesonide:DM-β-CD (1:10) Solid complex	67.7%	18.9	32.3%	39.6

It is evident that there is an endothermic signal at 260 °C on the physical mixture (Figure 1C), which correspond to budesonide's melting point (Figure 1A) that does not appear in the DSC of the solid complex (Figure 1D). The DSC was performed in the 1:1 ratio powders due to the fact that at higher DM-β-CD proportions the peak of budesonide is hidden by the DM-β-CD's endothermic response. For the particle characterization tests a budesonide:DM-β-CD powder ratio 1:10 was prepared, as justified below. It is assumed that if the complex was formed for the 1:1 ratio it will also be present in the 1:10 molar proportion.

Content uniformity of budesonide in the capsules filled with the solid complex and with the physical mixture

Budesonide content was determined in the capsules filled with budesonide:DM-β-CD solid complex and physical mixture (ratio 1:10). This proportion was used in order to achieve a suitable volume to fill the device's capsules. DM-β-CD worked in this case both as a complexation agent and as a filler.

The average of budesonide's content, (sd); (n) in the physical mixture was 204.9 μg ($\pm$ 9.35); (10) which corresponds to 102.5% ($\pm$ 4.67); (10) of the nominal dose (200 μg). The average assay of budesonide in the solid complex was 191.8 μg ($\pm$ 2.74); (10), which corresponds to 95.9($\pm$ 1.37); (10) of the nominal dose (200 μg). The results suggest that drug:CD complexes may help obtaining higher drug content uniformity in the dry powders for inhalation, often difficult to achieve in low drug dose mixtures.

Particle size distribution and fine particle fraction determination in the solid complex and physical mixture

The particle size distribution was measured in budesonide, DM-β-CD, budesonide:DM-β-CD physical mixture and solid complex, 1:10 ratio, using Aerosizer®LD (Figures 2A to 2D). Budesonide shows a normal and very narrow particle size distribution (95% below 2.0 μm), while DM-β-CD presents a very broad particle size range (from 1.0 to 12.9 μm). The physical mixture presents two different populations of particle sizes, which correspond to the ranges observed in the individual components. The complex powder presents a mono-modal and sharply distributed particle sizes (95% below 2.5 μm and 5% below 0.67 μm).

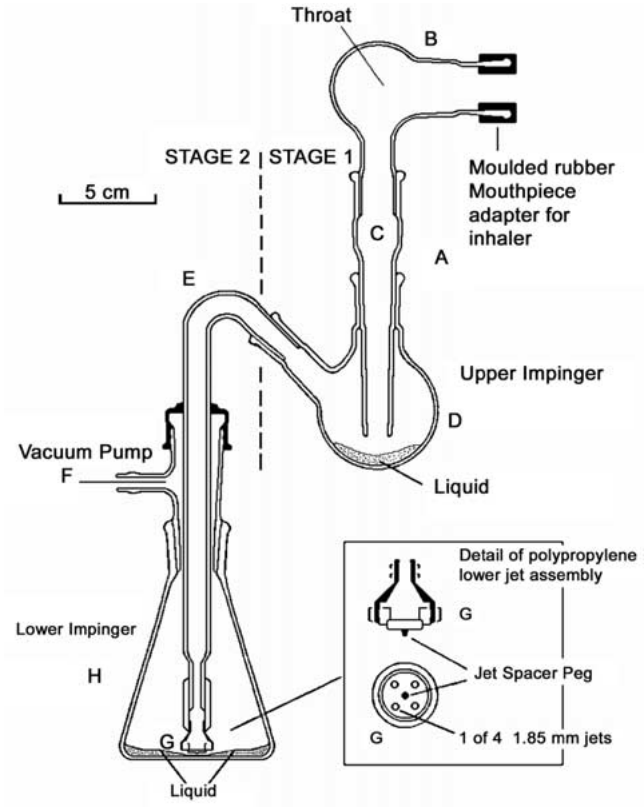

Figure 3. Schematic representation of the TSLI (Eur. Pharm. Apparatus A).

The lung deposition degree of both the physical mixture and solid complex was *in-vitro* assessed using the TSLI. The apparatus (Figure 3) consists of an upper stage (components A to D), which represents the upper respiratory tract and a lower stage (components E to H), which represents the lower respiratory tract. The emitted dose is separated according to particle size: greater than 5.8 μm (upper stage) and smaller than 5.8 μm (lower stage).

In order to be absorbed and exert its therapeutic effect more efficiently, drugs need to reach this latter area, also known as respirable fraction or fine particle fraction. Table 1 shows the amount of budesonide emitted from the inhaler and retained in the capsule, as a % of the nominal dose (200 μg). Table 2 presents budesonide's % of the emitted dose deposited in the lower and upper stage of the TSLI.

From the statistical analysis of the data obtained it is possible to conclude that while there is no statistically significant difference on the emitted dose of the physical mixture

and the solid complex at 95statistically significant higher mean content of budesonide reached the lower stage in the solid complex (67.7%), when compared to the physical mixture (39.8%), ($p = 0.005$). The results also show a very low reproducibility in the drug discharge on the solid complex, mainly in the non-emitted dose (rsd = 61.6%).

Conclusion

Budesonide is able to produce stable complexes with β-CD, DM-β-CD and HP-β-CD and presents the highest stability constant with DM-β-CD. The smaller and narrower particle size range and the statistically significant higher fine particle fraction obtained with the solid complex when compared to the physical mixture, suggest that using a spray-dried CD complex powder for pulmonary drug delivery may increase the drug's respirable fraction and consequently its therapeutic efficacy. The results obtained indicate a higher fine particle fraction than that referred in the literature for Pulmicort Turbuhaler® [14, 15]. In addition, the lack of reproducibility observed suggests that further work on the formulation is required, possibly in the spray- drying process conditions, to obtain a better powder flowability, hence achieving a higher reproducibility of the emitted dose.

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