European Journal of Pharmaceutics and Biopharmaceutics 72 (2009) 214–218

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Characterization and in vitro evaluation of the formoterol/cyclodextrin complex for pulmonary administration by nebulization

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ARTICLE INFO

Article history:
Received 9 July 2008
Accepted in revised form 22 October 2008
Available online 5 November 2008

Keywords:
Formoterol
Cyclodextrins
NMR
Nebulization efficiency

ABSTRACT

The aim of this work is to investigate the effect of cyclodextrin complexation on the pulmonary deposition of formoterol, a drug with a very poor aqueous solubility, after jet nebulization. Two types of cyclodextrins, a hydroxypropyl β cyclodextrin (Kleptose HP) and a polydispersed methyl β cyclodextrin (Crysmeb) were used. The interactions of formoterol with the cyclodextrins were studied by NMR. The aqueous cyclodextrin solutions containing formoterol were defined by their physicochemical properties in relation to nebulization capacity: density, surface tension and viscosity. Nebulization efficiency was evaluated by measuring droplet size, nebulization rate, quantity nebulized and nebulization time. The NMR ROESY spectra suggest that formoterol or a part of it is included inside the cyclodextrins. Densities and viscosities of the solutions tested are close to those of water; the lower surface tensions compared to water (53.7 and 56.7 vs 70 mN/m) favour the formation of small droplets. The aqueous solutions of cyclodextrins and formoterol studied can generate aerosols with a particle size that is compatible with pulmonary deposition. Respirable fraction values between 57.5% and 88 % were obtained when nebulizing the solutions with four nebulizers that differ geometrically. Nebulization rates varied from 0.19 to 0.47 g/min. Large quantities of drug nebulized over acceptable delivery times were observed. β-cyclodextrin derivatives can be used to formulate nebulizable solutions of formoterol. It is indispensable to define the appropriate nebulizers and operating conditions associated with the solutions to obtain adapted and reproducible activity.

1. Introduction

Pulmonary administration of drugs is usually intended for topical action and is an interesting alternative to systemic drug delivery [1]. The poor aqueous solubility and dissolution of a drug may be an absorption rate-limiting step in pulmonary drug delivery. Cyclodextrins have been used extensively as pharmaceutical excipients to increase the solubility of poorly water soluble drugs by the formation of an inclusion complex between the host cyclodextrin molecule and the guest drug molecule [2]. Moreover, studies have been shown to increase stability and bioavailability of drugs [3,4].

Cyclodextrins are composed of glucose units connected by α-1,4 glycosidic linkages to form a series of oligosaccharide rings. The native cyclodextrins comprise 6, 7 and 8 glucose units (α, β, γ, cyclodextrin, respectively). They have been chemically modified to improve their properties. The most important chemically modified cyclodextrins are hydroxypropyl and methylated β cyclodextrins that are much more soluble in water than the native β cyclodextrin. Hydroxypropyl β cyclodextrin can be used safely as a carrier for the parenteral delivery of drugs. It is cleared from the lung by being absorbed into the systemic circulation following administration in an aerosol [2,5]. Recently, the aqueous inclusion complexes of benzothiophene compounds with Hydroxypropyl β cyclodextrin in intravenous formulation, aerosol solution and oral formulation have been patented for the prevention of bone loss and lowering serum cholesterol levels [6].

The cyclodextrin solutions, used to enhance drug solubilities and to improve their administration into the lungs, could be administered into the lungs by nebulization. Little has been published concerning the pulmonary application of cyclodextrins, in particular, by jet nebulization. The effectiveness of nebulization depends on the site of aerosol deposition and on the proportion of drug deposited. This will be influenced by the characteristics of the aerosol, in particular, the size of the droplets emitted. These characteristics will depend on the formulation and on the administration conditions. Regarding the formulation, variables such as surface tension and viscosity may influence the fragmentation into droplets [7] and, therefore, affect output and particle size [8–10]. Regarding administration conditions, most of the time, the use of

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doi:10.1016/j.ejpb.2008.10.012

nebulizers is badly defined: the airflow, pressure and type of nebulizer is not specified for a given solution. However, it is of prime necessity to specify these parameters because they influence the particle size, the percentage of solution nebulized, and the nebulization time – that is to say, the nebulization efficiency [11,12].

The aim of this work was to investigate the effect of cyclodextrin complexation on the pulmonary deposition of formoterol, a drug with a very poor aqueous solubility, after jet nebulization. We also wanted to determine the optimal conditions of nebulization for the formoterol/cyclodextrin complex.

We first determined the physicochemical properties of the solutions of the formoterol/cyclodextrin complex: density, viscosity and surface tension. The complex formation was studied by NMR. To characterize the nebulized aerosol, we evaluated droplet size, nebulization rate, quantity of solution nebulized and nebulization time.

2. Materials and methods

2.1. Materials

Kleptose HP (hydroxypropyl β cyclodextrin, MW 1481) and Crysmeb (polydisperse methyl β cyclodextrin, MW 1190) were kindly supplied by Roquette Frères (Lestrem, France); formoterol fumarate; water for injectable preparations.

Three millilitres of each of the aqueous solution was nebulized with four different nebulizers (Table 1). A compressor for which airflow and pressure are adjustable supplied the air necessary to the nebulizer. The compressor capacities are 30 l/min and 10 bars. Pre-determined conditions of 16 l/min airflow and 2 bars pressure were applied to the nebulizer containing the complex solutions.

The nebulizers and the delivery conditions were determined in a previous study on cyclodextrin nebulization without drug [13]; it was the optimal nebulizers and operating conditions. Temperature and relative humidity were maintained constant at 20° and 40–45%.

2.2. Methods

2.2.1. NMR

All NMR experiments were carried out on a Bruker AVANCE 300 spectrometer operated at 300.09 MHz and equipped with a multinuclear z-gradient inverse probehead. In all experiments, the probe temperature was maintained at 298 K and standard 5 mm NMR tubes were used. NMR spectra were realised on a solution of 1 mM formoterol and the cyclodextrin (20 mM) in D_2O.

2D-ROESY (Rotating-frame Overhauser Effect Spectroscopy) spectra were recorded with a mixing time of 1 s during the spin-lock. 128 scans were collected for each of the 256 experiments.

2.2.2. Preparation of the formoterol/cyclodextrin complex for nebulization

Twenty-millimolar cyclodextrin and 200 μg/ml formoterol fumarate were added to water and shaken horizontally in a flask at 37 °C overnight.

Table 1
Nebulizers tested, manufacturers and addresses.

Nebulizer	Device description	Manufacturer
System 22	Small reservoir geometry, low dead volume	Medic Aid, Pagham, UK
Pari LC	Small reservoir geometry, low dead volume	Pari, Starnberg, Germany
Rotaneb NA 10	Large reservoir geometry, low dead volume	Europe Medical UK Ltd., Fleet, UK
RapidFlo	Small reservoir geometry, very low dead volume	Allersearch, Australia

2.2.3. HPLC analysis of the formoterol

The quantitative determination of fumarate formoterol was performed by HPLC using the method previously described by Akapo and Asif [14].The HPLC system consisted of a ProStar 230 pump (Varian, Middelburg, The Netherlands), an autosampler Prostar 410 (Varian), an UV–vis detector Prostar 325 (Varian) and a 150 × 4.6 mm i.d. column packed with 5 μm Pursuit C18 (Varian) which was maintained at 25 °C. The HPLC system was controlled by Galaxie software (Varian). The mobile phase (1 ml/min) consisted of ammonium acetate (50 mM, pH 5) and methanol in the ratio 65/35 v/v. The solvent of formoterol consisted of acetonitrile and water in the ratio 2/98 v/v. The formoterol eluted with a retention time of 11 min and was monitored at 210 nm. Standard calibration curves were prepared with 11 known concentrations of formoterol ranging from 0.05 to 20 μg/ml.

2.2.4. Characterization of the complex solutions

The solutions were defined by their physicochemical properties in relation to nebulization capacity: density, surface tension, viscosity. The results are the mean of three replicate measurements.

2.2.4.1. Density. Density was measured by using an Anton Paar type DMA45A densitometer (Anton Paar SAS, Courtaboeuf, France).

2.2.4.2. Surface tension. The surface tension measurement method consists in measuring the force that has to be exerted on a platine/irridium stirrup piece, which is in contact with the solution surface, to stretch the interfacial liquid film. In our case, the test body was a frame. It was made with a Lauda TD1 tensiometer (Prolabo, Paris, France), the measuring range of which is 0–100 mN/m, precision 0.1 mN/m and sensitivity 0.001 mN/m.

2.2.4.3. Viscosity. The viscosity of the solutions, the behaviour of which is Newtonian, was measured with a Micro Ostwald capillary viscosimeter (Schott-Geräte, Germany) following the method described in the European Pharmacopoeia [15].

2.2.5. Evaluation of nebulization efficiency

Different parameters were evaluated to qualify the nebulization efficiency.

2.2.5.1. Droplet size. The aerosol size distribution emitted from cyclodextrin solutions was determined with a laser size analyser Mastersizer X (Malvern, Orsay, France) using Mie theory. The focal length of the lens was 300 mm. The solution was directly nebulized in the laser beam. After repeated testing, the measurement variation is 2.4%. The results are expressed as the percentage of droplets between 0.5 and 6.4 μm and the volume median diameter. We checked that particle size did not change over the period of nebulization.

The laser diffraction technique for particle size measurement in the aerosol clouds has been used in several studies of medical nebulizers [9,16–18]. It has been concluded that the technique is robust and reliable, and that it measures size parameters relevant to the clinical situation [16,19]. With the laser size analyser, distribution is made according to particle volume. With an impactor, separation depends on the kinetic energy of the droplet, which is itself a function of the mass. In fact, of all aerosols to be measured, only aqueous droplets from nebulization are spherical and approach unit density. Therefore, their median diameter measured by laser diffraction technique equals the median aerodynamic diameter obtained by impaction methods [16]. We obtained similar results for these two diameters in a previous work about nebulization of a protein solution [20].

2.2.5.2. Nebulization rate. The mass loss after 3 min of nebulization was used to determine the nebulization rate (g/min).

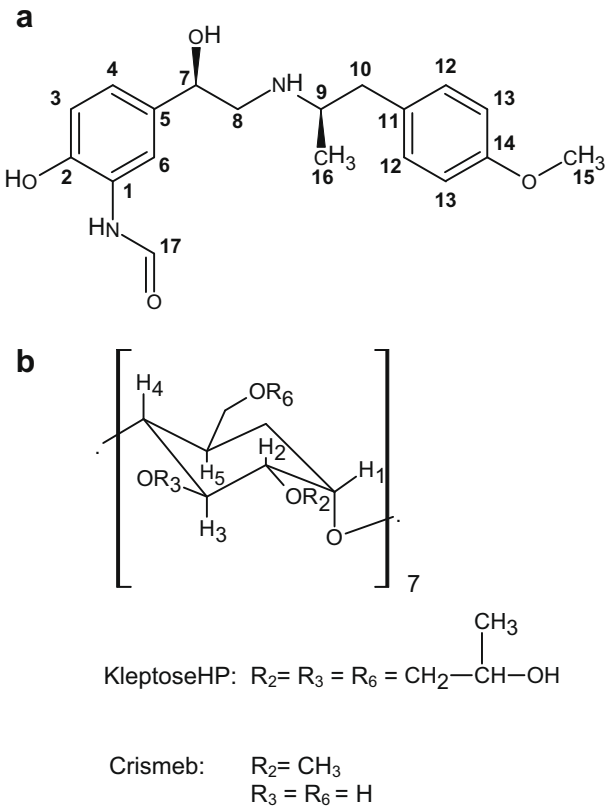

Fig. 1. Structures and assignments of the hydrogen atoms of the formoterol (a) and the cyclodextrins (b).

2.2.5.3. Quantity of solution nebulized. The amount of solution released as aerosol was obtained by subtracting the amount remaining in the nebulizer from the mass initially placed in the system. It was expressed in percentage. The amount remaining in the nebulizer is the "dead volume" which is lost for the patient.

2.2.5.4. Quantity of formoterol nebulized. The amount of formoterol released as aerosol was obtained by subtracting the amount remaining in the nebulizer from the amount initially placed in the system. The amount remaining in the nebulizer was determined by HPLC after rinsing the nebulizer with 50 ml of acetonitrile/water (2/98 v/v).

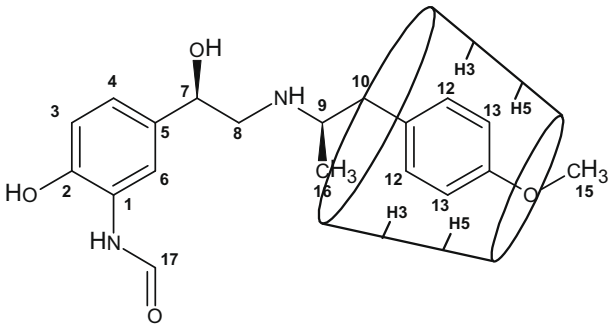

Fig. 3. Proposed model for the inclusion of formoterol in Crysmeb.

2.2.5.5. Nebulization time. This parameter is important for patient compliance and must be taken into consideration when evaluating the performance of nebulizers. The end of nebulization was considered when no more aerosol is produced. This aerosol was not tapped to assist aerosol production.

2.2.5.6. Statistical analysis. A statistical ANOVA *F* test was applied to the results. Double way ANOVA test was used considering the type of cyclodextrin and the type of nebulizer.

3. Results and discussion

Structures and assignment of the hydrogen atoms of the formoterol and cyclodextrins (Kleptose HP and Crysmeb) are presented in Fig. 1.

Two dimensional ROESY spectroscopy is capable of revealing spatial relationships among protons in a molecule or in a complex of molecule. This experiment utilizes the dipolar interaction between protons at distances less than 5 Å.

The partial 2D-ROESY spectrum of the formoterol/Crysmeb is reported in Fig. 2. First, intramolecular interactions are detected H_{10}–H_{12}, H_8–H_6, H_8–H_4, H_{15}–H_{13}, H_7–H_6 and H_7–H_4. Second, intermolecular interactions (marked with an horizontal arrow in Fig. 2) were detected between H_{12} and H_{13} with cyclodextrins protons (H_3, H_5 and/or H_6). The H_3 and H_5 of the cyclodextrin being located

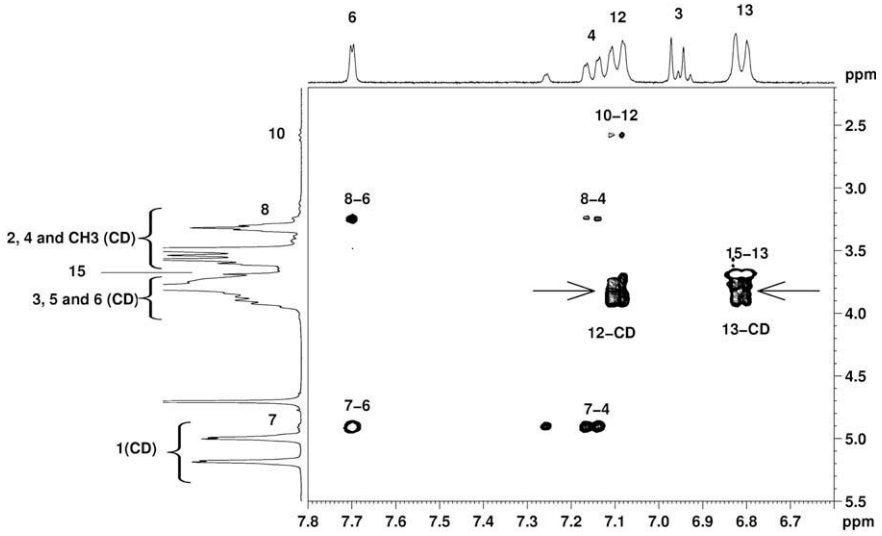

Fig. 2. Partial ROESY spectrum of formoterol/Crysmeb complex.

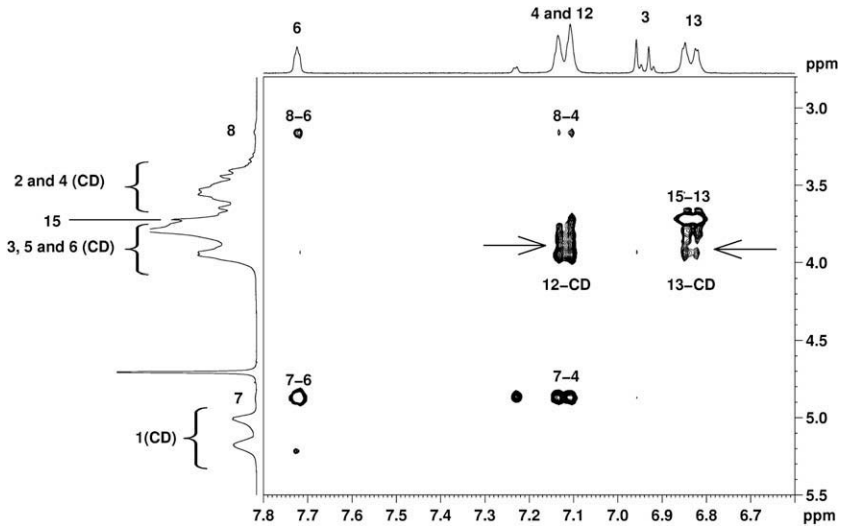

Fig. 4. Partial ROESY spectrum of formoterol/Kleptose HP complex.

Table 2
Physicochemical properties of the aqueous solution of formoterol (200 μg/ml) and cyclodextrin (20 mM).

Cyclodextrin associated with the formoterol	Density (g/cm^3)	Viscosity (mPa s)	Surface tension (mN/m)
Kleptose HP	1.004	1.16	53.7
Crysmeb	1.004	1.18	56.7

inside the cyclodextrin cavity, these results show the formoterol complexation through the disubstituted aromatic ring into the hydrophobic cavity of cyclodextrin. The inclusion complex model is presented in Fig. 3.

The partial 2D-ROESY spectrum of the formoterol/Kleptose HP is reported in Fig. 4. Cross peaks between formoterol (H_{12} and H_{13}) and cyclodextrin protons are observed. This confirmed complexation of formoterol disubstituted aromatic ring into the Kleptose HP.

Table 2 presents the physicochemical properties of the aqueous solutions containing the formoterol/cyclodextrin complex. Densities and viscosities are close to those of water (1 g/cm^3, 1 mPa s). The lower surface tensions compared to water (70 mN/m) are in favour to the formation of small droplets [9,21]. The density of 1 g/cm^3 makes it possible to compare the size obtained with the laser size analyser and the one that could be obtained with a cascade impactor as discussed in the Section 2.2.5.1.

Fig. 5 presents the influence of the cyclodextrin and of the nebulizer on the droplet size. We noted the volume median diameter and the percentage of droplets between 0.5 and 6.4 μm. The 6.4 μm threshold was retained because when using the Twin impinger as an impactor, the aerosol fraction collected in the lower part is the fraction below 6.4 μm. This fraction is the fine particle fraction that is to say the portion of the inhaler output that may be expected to correlate with the fraction of the drug dose that penetrates the lung during inhalation [22].

The results are comparable for the two cyclodextrins. The percentage of droplets between 0.5 and 6.4 μm and the volume median diameter differ according to the nebulizer considered ($p < 0.01$ and 0.1, respectively). This confirms the influence of the nebulizer design on aerosol size.

Fig. 6 presents the influence of the cyclodextrin and the nebulizer on the nebulization rate. Here again, there is no difference between the two cyclodextrins but there is an influence of the nebulizer ($p < 0.005$). The Pari LC nebulizer presents the lower nebulization rate; this is also the one that gives the smaller percentage of droplets between 0.5 and 6.4 μm. Its geometry is more efficient in retaining the larger droplets that are not emitted and that drain back into the reservoir for re-nebulization. Only the smaller particles are carried out of the nebulizer, but this will increase the nebulization time. A compromise must be found between acceptable droplet size and nebulization time.

Results are not significantly different (ANOVA) for the two cyclodextrins tested. The hydroxypropylated and methylated

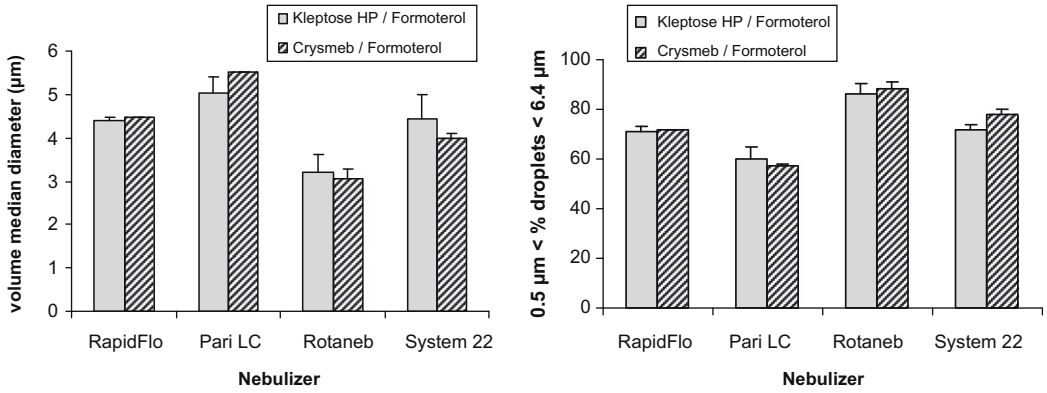

Fig. 5. Size of the droplets obtained from the aqueous solutions of formoterol (200 μg/ml) and cyclodextrins (20 mM) ($n = 3$).

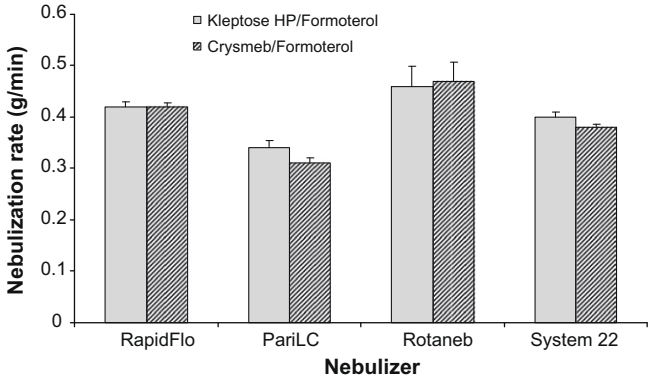

Fig. 6. Nebulization rates obtained with the aqueous solutions of formoterol (200 μg/ml) and cyclodextrins (20 mM) (n = 3).

Table 3
Quantity of solution and formoterol nebulized and nebulization times obtained with the aqueous solution of Crysmeb (20 mM) and formoterol (200 μg/ml) (n = 3).

Nebulizer	Dead volume (ml)	% Solution nebulized	% Formoterol nebulized	Nebulization time (min)
RapidFlo	0.46 ± 0.06	84.65 ± 2.10	67.49 ± 2.01	7 ± 0.6
Pari LC	1.10 ± 0.02	63.54 ± 0.84	47.68 ± 2.03	9 ± 0.5
Rotaneb	0.98 ± 0.07	67.18 ± 2.61	49.70 ± 2.99	6 ± 0.7
System 22	0.82 ± 0.04	72.74 ± 1.54	53.22 ± 4.09	7.5 ± 0.6

cyclodextrins associated with the formoterol have the same behaviour. The nature of the substituent of the native cyclodextrin does not seem to have an influence for this application. So, for the quantity of solution nebulized and nebulization time, we only present those obtained with the Crysmeb cyclodextrin (Table 3).

The quantity of solution nebulized varies from 64% to 85% according to the nebulizer considered. Nebulization times are in agreement with nebulization rates. Contrary to the Pari LC nebulizer, the Rotaneb nebulizer presents the higher nebulization rate and the shorter nebulization time. But its dead volume is high (1 ml) and represents about 30% of the solution placed in the nebulizer. The RapidFlo and System 22 nebulizers show similar nebulization times but the dead volume is lower in the case of the RapidFlo.

The percentage of drug nebulized is lower than the percentage of solution nebulized because of the concentration that occurs during nebulization. During nebulization, solvent output is considerable, leading to an increase in drug concentration in the liquid remaining in the chamber of the nebulizer [23]. The percentage of formoterol nebulized varies from 48% to 68% according to the nebulizer considered. The RapidFlo nebulizer that presents a low dead volume shows the higher percentage of formoterol nebulized. Percentages of drug nebulized are in agreement with dead volumes.

The Rapidflo presents the lower dead volume (0.46 ml), a high nebulization rate (0.42 g/min), the higher percentage of formoterol nebulized, a satisfying nebulization time (6 min) and a droplet size suitable for an administration deep into the lungs (about 72% droplets between 0.5 and 6.4 μm).

4. Conclusion

β-Cyclodextrins derivatives can be used to formulate nebulizable solutions of formoterol. NMR studies and ROESY spectra show the complexation of formoterol with the cyclodextrins studied.

We characterized the aerosols obtained by jet nebulization under different operating conditions. The use of different nebulizers results in a variable efficiency. The aqueous cyclodextrin/formoterol solutions studied can generate aerosols the particle size of which is compatible with pulmonary deposition. High quantities nebulized during acceptable nebulization times can be obtained.

Aerosol properties are shown to be the result of an association formulation/equipment, the latter taking into consideration nebulizer geometry and operating conditions. It is indispensable to define using conditions by size measurement and quantity nebulized for any given formulation.

Acknowledgements

The 300 Hz NMR facilities were funded by the Région Nord-Pas de Calais (France), the Ministère de la Jeunesse, de l'Education Nationale et de la Recherche (MJENR) and the Fonds Européens de Développement Régional (FEDER).

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