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Study of β-cyclodextrin and ethylated β-cyclodextrin salbutamol complexes, in vitro evaluation of sustained-release behaviour of salbutamol

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Abstract

Solid complexes of salbutamol with β-cyclodextrin (β-CD) and different batches of ethylated β-cyclodextrin (Et-β-CD) in a 1 : 1 molar ratio were prepared by the freeze-drying method. Inclusion complexation of salbutamol with all the CD used was confirmed in the solid state by differential scanning calorimetry (DSC), and in aqueous solution by circular dichroism analysis and electrospray mass spectrometry (ES-MS). The dissolution rate of salbutamol from the inclusion complexes was determined in water. We discuss the behaviour of the dissolution profiles obtained, since the Et-β-CD complexes displayed a slower release of salbutamol compared with the β-CD complex.

Keywords: β-Cyclodextrin; Ethylated β-cyclodextrin; Salbutamol; Inclusion complexes; Circular dichroism; Differential scanning calorimetry; Electrospray mass spectrometry; Dissolution behaviour

1. Introduction

Native cyclodextrins (CDs), products of starch bioconversion, are torus-shaped molecules, made up of different numbers of α-(1,4) linked D-glucopyranose units, α-, β- and γ-CD encompassing respectively six, seven and eight of these units. The CD cavity exhibits a hydrophobic character, whereas the exterior of the molecule is hydrophilic. The CDs have been shown to form a variety of complexes in which guest molecules are

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trapped entirely or at least partially by the hydrophobic portion. In addition to their polarity, the ability of the molecules to penetrate the CD host is determined by its size and shape, and this inclusion leads to changes in the physicochemical properties of the guest molecules. Therefore, CDs are useful for a reduction in the undesirable properties of drug molecules. Complexation may suppress volatility and unpleasant odours or tastes associated with the drug. Moreover, the cavity provides a protection, and may help to avoid problems of incompatibility with other drugs or excipients in a formulation, and also reduces the local irritance or haemolysis of a drug (Duchêne et al., 1986; Szejtli, 1984, 1991).

Recently, many kinds of chemically-derivated CDs have been prepared to extend the physicochemical and inclusion capacities of natural CD as multifunctional drug carriers. The hydroxyl groups of CD are available as starting points for structural modification, without eliminating the central cavity available for guest accommodation (Duchêne and Wouessidjewe, 1990; Uekama et al., 1991). The CD derivatives, especially methylated (Pitha et al., 1986; El-Gendy and El-Gendy, 1993) and hydroxypropylated (Uekama, 1985; Vollmer et al., 1993; Arias et al., 1995) have been studied extensively, because of their usefulness for improving the solubility, dissolution rate and bioavailability of poorly water-soluble drugs. However, little work has been carried out on ethylated cyclodextrins (Et-CD). Some work is available, particularly on Et-β-CD that acts as sustained-release carrier of water-soluble drugs (Uekama et al., 1987, 1989; Umemura et al., 1990; Kawahara et al., 1992).

Salbutamol (albuterol) is a β_2-selective adrenoreceptor agonist which accounts for its pronounced bronchodilatory, cardiac, uterine and metabolic effects. It was chosen as the drug model for this study. Because of its short biological half-life, it seems to be interesting in prolonging the duration of the therapeutic action of salbutamol, in order to improve patient compliance. Furthermore, in a recent work, Cabral Marques et al. (1990a) showed that the cavity size of β-CD or its derivatives was optimal for entrapment of the salbutamol molecule. For this purpose, we examined in this study the feasibility of using complexation with Et-β-CD to produce a sustained release of salbutamol.

The inclusion reaction can occur in various ways (Hirayama and Uekama, 1986). It generally takes place in aqueous or organo-aqueous solution (coprecipitation), or in a slurry (kneading method), by heating in a sealed container, or by simple grinding, and also by freeze-drying. Freeze-drying was shown to be suitable for substances which are water soluble, or which decompose by drying. It produces a powdered sample in a very good yield (Jones et al., 1984). Hence, this method was chosen to prepare complexes of salbutamol and Et-β-CD. For this study we have used five different batches of Et-β-CD, obtained by different synthesis methods.

Several methods are currently used for the characterization of CD guest complexes such as differential scanning calorimetry (DSC) (Castelli et al., 1989), nuclear magnetic resonance (Ueda and Nagai, 1980; Djedaïni et al., 1990), circular dichroism (Buss, 1992), X-ray (Hirayama et al., 1994), infrared (Wouessidjewe et al., 1989) and mass spectroscopy (Drabowicz et al., 1994). Amongst these methods, we have chosen to characterize our complexes in the solid state by DSC, and in the liquid phase by circular dichroism and with a recently developed method, the electrospray ionization mass spectrometry (ES-MS) analysis.

Finally, the study of the dissolution rate of salbutamol CD complexes including all the CDs investigated was carried out in water, and the different behaviours observed are discussed comparatively.

2. Materials and methods

2.1. Materials

β-CD (Kleptose®) was supplied by Roquette (Lestrem, France).

Et-β-CD Number 1 was purchased from Chinoin (Budapest, Hungary).

Et-β-CD Numbers 6, 9A, 10 and 10A, were provided by courtesy of Orsan CRB (Les Ulis, France).

The detailed process of synthesis and the chemical characterization of all Et-β-CDs used will be reported elsewhere in a future publication. Nevertheless, the average degrees of substitution (DS) of ethyl groups, which were determined by ES-MS (Sorokine et al., 1992), and their aqueous solubility are summarized in Table 1.

Salbutamol was obtained from Glaxo Laboratories (Evreux, France). It was used without further purification.

Other chemicals and solvents were of analytical reagent grade. The deionized double-distilled water, which was prepared in our laboratory, was used throughout the study.

2.2. Freeze-drying preparation method

Salbutamol (5 mg) and β-CD (24 mg) or Et-β-CD (32 mg), corresponding to a 1 : 1 molar ratio, were dissolved in distilled water and in ethanol : water (50 : 50) for the Et-β-CD No. 1, and mixed at 25°C for 6 h, protected from light, before freeze-drying. Freeze-drying was performed in an Alpha I/5 Christ freeze-drier (Osterode, Germany), always in the dark.

The physical mixtures were prepared by mixing the amounts of freeze dried salbutamol and CD in a 1 : 1 molar ratio in a mortar.

2.3. Characterization methods

2.3.1. Differential scanning calorimetry

DSC measurements were carried out by using a Perkin-Elmer DSC 7 (St Quentin en Yvelines, France) with a data analysis system. The sample (5 mg) was heated at a scanning rate of 10°C/min over the temperature range 0–200°C. The heat of the fusion was calibrated with Perkin-Elmer standards, indium (purity 99.999%, melting point 156.60°C) and zinc (purity 99.999%, melting point 419.47°C).

2.3.2. Circular dichroism

Circular dichroism spectra were recorded with a Jobin Yvon Mark V dichrograph. Complexes were studied by circular dichroism and expressed by $\Delta\epsilon$, i.e. the differential molar dichroic absorption coefficient (M^{-1} cm^{-1}).

2.3.3. Electrospray mass spectrometry

ES-MS measurements were carried out on a VG-TRIO 2000 electrospray mass spectrometer VG Biotech (Altrincham, UK), a quadrupole instrument with an atmospheric pressure electrospray module. The extraction cone voltage was 50 V. Complexes were introduced into the source at a flow rate of 5 μl/min. The calibration was realized with horse myoglobin (1695.5 Da).

2.4. Dissolution study

The sample powders containing the complex (equivalent to 5 mg of salbutamol), were tabletted in a single-punch tabletting machine (Frogerais OA, Vitry, France). The tablets obtained were convex faced (diameter 3 mm) and the hardness, measured on a Schleuniger hardness tester, was 4 kp. The release rate of salbutamol was carried out at 37°C by using the USP XXII paddle method, Sotax AT6 apparatus, OSI (Maurepas, France). The tablet was immersed in 500 ml of water, in the dark, and rotated at a constant speed of 50 rotations/min. At appropriate intervals, an aliquot of 2 ml was withdrawn, and high-performance liquid chromatography (HPLC) was employed for the determination of salbutamol. The HPLC conditions were as follows: a chromatograph, pump and Waters-type detector equipped with a 486 Waters UV monitor (Milford, Massachusetts, USA). The column was μ-bondapack NH2 125 A, 10 μm, 3.9 mm i.d. × 150 mm, Waters (Milford, Massachusetts, USA). The mobile phase was isopropanol: 0.015 M, ammonium acetate (1 : 6). The flow rate was 1 ml/min, with detection at 276 nm.

3. Results and discussion

3.1. Characterization of the complexes

The thermal behaviour of the CD inclusion compound was studied by DSC, in order to confirm the formation of a solid complex. When guest molecules are incorporated in the CD cavity or in the crystal lattice, their melting, boiling and sublimation points usually shift to a different

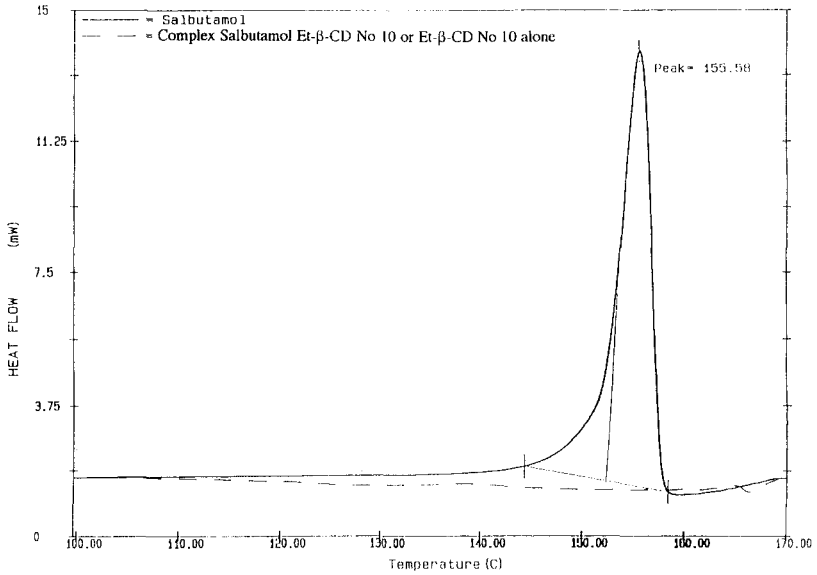

Fig. 1. DSC thermograms of freeze-dried salbutamol and its complex with Et-β-CD No. 10 obtained by the freeze-drying method.

temperature or disappear within the temperature range of which the CD lattice is composed (Cabral Marques et al., 1990a). Fig. 1 shows a typical example of powder DSC thermograms. The endothermic peak (155.8°C) of freeze-dried salbutamol was due to the melting of salbutamol crystals. This peak did not change in the physical mixture. However, this endothermic peak disappeared in the case of a freeze-dried inclusion compound, due to the interaction of salbutamol with Et-β-CD, which suggests that a complex was formed in a solid state.

The interactions in the liquid phase were further examined by circular dichroism spectroscopy. The induced circular dichroism of the complexes is generally characterized by their sign, magnitude, and wavelength of the location. According to the symmetry rule, the sign of the induced Cotton effect depends upon the spatial relationship between the asymmetrical centre and the perturbed chromophore, whereas the magnitude of the intrinsic Cotton effect seems to depend on the rigidity of the complex formed (Chun and Yun, 1993). Fig. 2 shows typical circular dichroism spectra of salbutamol in the absence and presence of Et-β-CD. Salbutamol exhibits a band with a maximum at 276 nm. This effect disappears with the complex, showing a strong interaction between the salbutamol and the Et-β-CD. Furthermore, the higher induced circular dichroism with Et-β-CD apparently suggests that the chromophore of salbutamol could be located in the cavity of the CD derivatives.

Electrospray mass spectrometry (ES-MS) is a soft method of ionization for non-volatile and thermolabile molecules (Metzger et al., 1991),

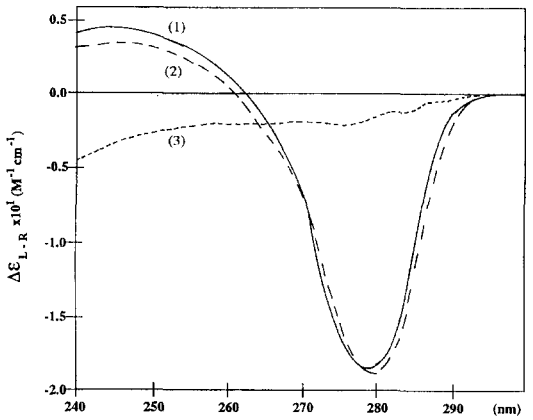

Fig. 2. Circular dichroism spectra of salbutamol in the presence and absence of Et-β-CD No. 6. (1) Salbutamol, (2) physical mixture, (3) complex.

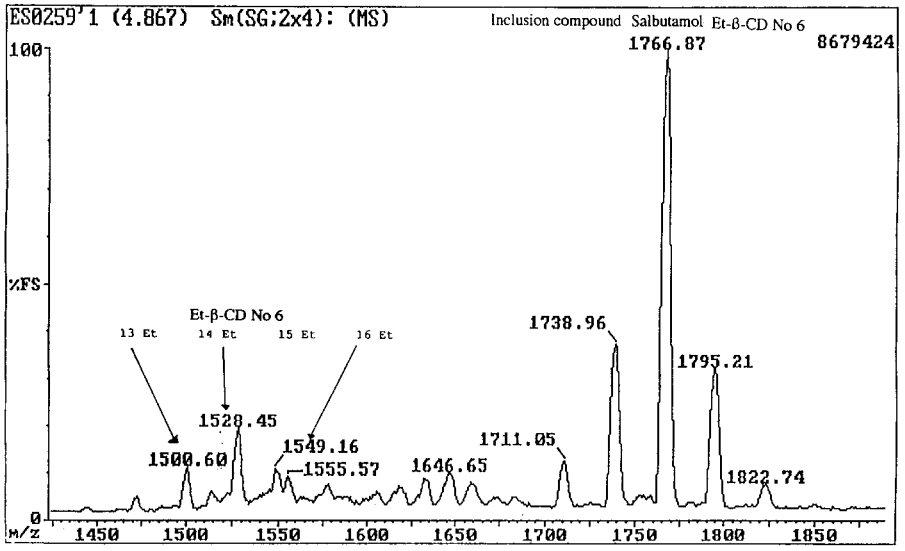

Fig. 3. ES-MS spectra of the inclusion complex salbutamol Et-β-CD No. 6.

mainly used for the characterization of peptides, proteins and enzymes. Recently, ES-MS appears to be a powerful, accurate and reliable analytical method for CD and inclusion complexes. Bates et al. (1993) have shown that ES-MS is a good method to examine directly CD guest complex formation. Sorokine et al. (1992) also assumed that this method enables a better understanding of the inclusion mechanism and derivation of the stoichiometry. Fig. 3 shows an example of ES-MS spectra of inclusion compounds of Et-β-CD with salbutamol. Since this method hardly induces fragmentation, we can identify the inclusion compound in the 1 : 1 molar ratio.

Finally, these characterization analyses have shown a strong interaction between salbutamol and Et-β-CD, which suggests the formation of inclusion compounds. These data are in accordance with Cabral Marques et al. (1990b), who concluded that β-CD and methylated β-CD form an inclusion complex with salbutamol, and the probable stoichiometric ratio of the complex was 1 : 1. Therefore, the authors also suggested that the phenyl moiety of the salbutamol was included in the β-CD cavity.

3.2. Sustained-released study

The dissolution curves obtained for the salbutamol β-CD or different Et-β-CD complexes in water at 37°C are shown in Fig. 4. The freeze-dried salbutamol exhibited very fast dissolution in water, 100% within the first minute of the test. All the release profiles of the complexes represent the mean of three replicate values, with a standard

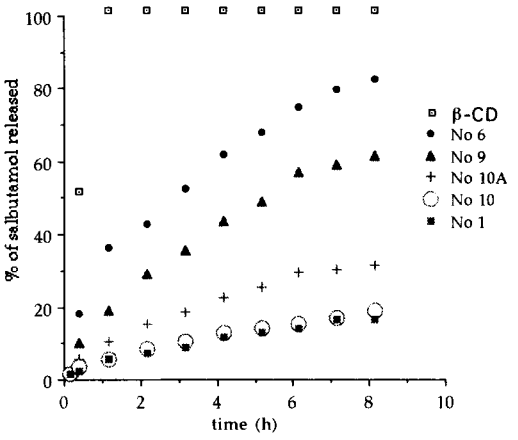

Fig. 4. Release profiles of salbutamol with β-CD and the different Et-β-CDs.

deviation less than 5%. It is obvious that the dissolution rate of salbutamol is significantly sustained by the Et-β-CD complex formation. Indeed, a total release is observed after 1 h with the natural β-CD, whereas this state is not yet reached after 8 h with the Et-β-CD No. 6. Furthermore, drug release was markedly delayed by complexation with the other Et-β-CD, in decreasing order from No. 6 < 9A < 10A < 10 < 1.

Hiriochi et al. (1990) have explained the sustained release of the diltiazem by the poor aqueous solubility of Et-β-CD. In this study, this hypothesis is in harmony with Et-β-CD No. 1 complexes, but cannot be applied to the other Et-β-CDs. Indeed, Et-β-CD No. 6 shows a higher aqueous solubility than β-CD, and the solubilities of No.s 9A, 10A and 10 do not exhibit a big enough difference to explain the significant sustained release obtained.

The binding constant (K) of the complex is the other important parameter to consider in the inclusion mechanism. This constant is a function of the good fit of the guest molecule inside the host molecule, and, if it is of sufficient magnitude, slow dissociation (i.e. drug release) may then occur. For this reason, we determined the binding constants between salbutamol and the different Et-β-CDs used (Lemesle-Lamache et al., in press) and compared them with that of the β-CD complex. We found that the salbutamol presents a higher binding constant with Et-β-CD than with β-CD. Furthermore, we obtained different values of binding constants for each of the salbutamol Et-β-CD complexes. These values are in the increasing order of $K1 > K10 > K10A > K9A > K6$ (Fig. 5) and they are in accordance with the profiles of sustained release shown in Fig. 4. Fig. 5 shows the ratio between the electrophoretic mobility of the salbutamol with 5 mM of CD ($\mu_{ep\ (5)}$) and the electrophoretic of the salbutamol alone ($\mu_{ep\ (0)}$). This ratio reflects the extent of the affinity between salbutamol and CD.

Considering all these observations, we suggest it is likely that the sustained release of salbutamol is due to its stronger complexation with the ethylated derivatives compared with β-CD. Thus, the ethylation of the hydroxyl groups may change the nature of the host–guest interactions. These ethylations may expand the hydrophobic region of the CD cavity, and enhance the binding of the substrate by means of the hydrophobic effects. But we also notice that over the whole range of Et-β-CDs used, average DS has not been very different from one to another (Table 1). However, we cannot correlate these DS values with differences in the sustained profiles obtained. The Et-β-CD No. 1 and Et-β-CD No. 6 which have the same DS value exhibited the greatest difference in ways of release. Therefore, we can also agree with Müller and Brauns (1986), who worked on methyl derivatives, that not only the degree of substitution, but also the substitution pattern of the CD derivatives, influence the complexation behaviour.

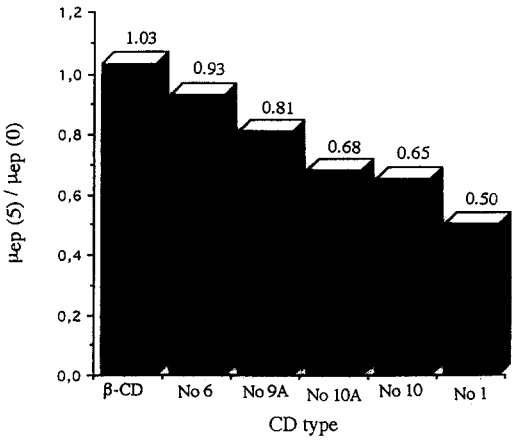

Fig. 5. Comparison of $\mu_{ep\ (5)}/\mu_{ep\ (0)}$ ratios obtained for β-CD and the different Et-β-CDs. Conditions: 40 mM citrate phosphate buffer pH 7, urea 8 M, 25°C, 15 kV.

Table 1
Average degree of substitution (DS) and aqueous solubilities of Et-β-CD

Et-β-CD No.	DS	Solubility g/l	
		25°C	37°C
1	1.98	0.4	0.1
6	1.98	38	34
9A	1.91	24	30
10	2.12	30	17
10A	2.06	26	13
β-CD	–	19	23

With a desired Et-β-CD derivative, which is well-defined from a chemical standpoint, and with well-known physicochemical characteristics, it is possible to obtain complexes with the required drug release.

4. Conclusion

We have shown that the freeze-drying method is simple and suitable for obtaining an inclusion complex of salbutamol with β-CD and Et-β-CD. The different sustained-release behaviours obtained suggest that both the solubility and the dissociation of the complexes are responsible for the dissolution rates of the drug. The results indicate that Et-β-CD may be considered as a good carrier for the sustained release of salbutamol.

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