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Determination of the binding constant of salbutamol to unmodified and ethylated cyclodextrins by affinity capillary electrophoresis

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Abstract

This paper describes a capillary electrophoretic method for the accurate determination of the binding constants (K) for inclusion complexes of β-cyclodextrin (β-CD) and ethylated β-cyclodextrin (Et-β-CD) with salbutamol. The model is designed to compensate for the change in viscosity caused by CD addition, which was shown to greatly affect the measurements. The influence of several operating parameters, e.g. temperature, pH of the running buffer and voltage, on the binding constants is discussed. The values for K obtained for β-CD and Et-β-CD are compared, and it is concluded that Et-β-CD has a greater affinity for salbutamol than β-CD. For the case of limited modified-CD solubility, a method is proposed to estimate the affinity of salbutamol for the CD. Finally, we demonstrate that the described method can efficiently predict the release-rate of a drug complexed with a given CD.

Keywords: Capillary electrophoresis; Binding constants; Salbutamol; Cyclodextrins

1. Introduction

Cyclodextrins (CDs) are cyclic non-reducing oligosaccharides with truncated cylindrical molecular shapes and are composed of six, seven or eight glucopyranose units (α-, β- and γ-CD, respectively) [1]. Their outside surfaces are hydrophilic, whereas their cavities are hydrophobic. Due to their particular conformation, CDs are able to enclose various kinds of drugs in their inner hydrophobic cavity, leading to changes in the physicochemical properties of the drugs [2]. The inclusion of various guest molecules is determined primarily by the size of the cavity of the CD, while the stability of the inclusion complex formed depends on the fit between the compound and the cavity. The cavity size of β-CD, which is intermediate between those of α-CD and γ-CD, is the most efficient host for many kinds of drugs.

The hydroxyl groups of the CDs are available as starting points for structural modification, and various functional groups have therefore been incorporated into CD molecules, with the aim to change both the polarity and the shape of the cavity [3–5]. Derivatization of CDs has also been

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used to increase their solubility in water, and to increase the stability of the host–guest complexes. Among the various derivatized CDs reported, ethylated cyclodextrins (Et-CDs) have been shown to sustain the release of water-soluble drugs following subcutaneous [6] or oral administration [7]. However, little work has been devoted to Et-β-CD [8–12]. The aim of the present study was therefore to gain an understanding as to how the sustaining of drug release in the presence of a given CD can be explained by the stability of the complex formed. Two batches of Et-β-CD (called 6- and 10-Et-β-CD), obtained by a synthetic route, were investigated.

Salbutamol was chosen as the drug model: it is a β-adrenoceptive receptor stimulant, widely used to produce bronchodilation. It is composed of a saligenin group, an amino alcohol portion containing a chiral centre and a tertiary butyl group. The structure of salbutamol is shown in Fig. 1.

In order to prolong the duration of the therapeutic action of salbutamol and to improve patient compliance, we previously tried to develop a sustained-release form with the ethylated derivatized β-CD form. Recently, we reported our findings on the use of Et-β-CD instead of β-CD to achieve a slower release of salbutamol [13]. When complexed with β-CD, 60% of salbutamol was released within 15 min, whereas 4 h was required for the same release with 6-Et-β-CD. Total release was observed after 1 h with β-CD, whereas this was only reached after 8 h with 6-Et-β-CD. With 10-Et-β-CD, only 20% of the salbutamol was released after 8 h. These results were interpreted to reflect a higher affinity between Et-β-CD and salbutamol compared with β-CD.

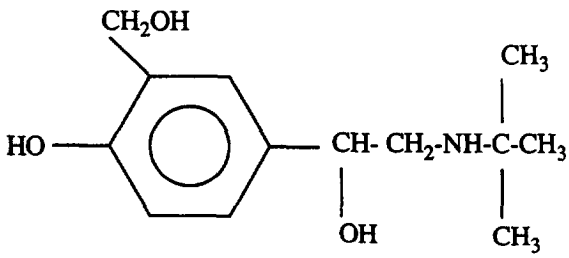

Fig. 1. Structure of salbutamol.

In an attempt to validate this hypothesis, we developed an affinity electrophoresis capillary method (AEC) to determine the binding constants of β-CD and Et-β-CD with salbutamol. Although previous works have described the use of CDs in capillary electrophoresis (CE) to achieve enantioseparations [14–23], various recent reports have demonstrated that CE can also be an attractive mode to study receptor–ligand interactions and to determine the binding constants of the complexes formed [24–30]. Most of the work done using CDs as a potential host for complexation with drugs has focused on α-, β-, γ-CD and on their dimethyl or hydroxypropyl derivatives [15]. However, to date, there are no reports on Et-CD as a host agent.

In the present paper, we first examine the influence of several parameters, such as temperature, buffer pH and applied voltage, on the binding constants K. The viscosity of a CD-containing solution, which is related to the CD concentration, was shown to affect the measurements significantly. Thus, to obtain accurate values of K, we investigated what would be the most suitable correction factor to compensate for the change in viscosity in the running buffer observed upon CD addition. Finally, the affinities of salbutamol for β-CD and Et-β-CD were compared, and the results were shown to be in good agreement with the data obtained on the release rate of this drug upon the addition of different types of CDs.

2. Experimental

2.1. Apparatus

Capillary electrophoresis was performed using a Beckman P/ACE 2100 system equipped with a capillary cartridge allowing efficient temperature control. A fused-silica capillary with an effective length of 50 cm (total length 57 cm) and 75 μm I.D. was used for the separation. Samples were introduced into the capillary by hydrodynamic injection for 3 s. UV detection was employed at a wavelength of 280 nm. Before each analysis, the capillary was rinsed under pressure with 0.1 M

NaOH for 5 min and then equilibrated with the running buffer for 5 min. After analysis, the capillary was flushed with distilled water for 3 min to avoid precipitation of CD in the capillary. The electroosmotic flow (EOF) was monitored by the migration time of the peak corresponding to water. Calculation of the binding constants was performed using the Minimum 1.8 program (Otago University, New Zealand) with a non-linear least-squares curve fitting procedure.

2.2. Materials and chemicals

β-CD was obtained from Roquette (Kleptose; Lestrem, France). Two Et-β-CD batches, called 6 and 10, obtained by a synthetic route, were provided by Orsan CRB (Les Ulis, France).

Salbutamol was a generous gift from Glaxo Laboratoires (Evreux, France). It was used without further purification. Salbutamol [pK_{a1} (phenol) = 9.4, pK_{a2} (amine) = 10 at 25°C], which has a chiral centre on its amino alcohol groups, was used as the racemate mixture.

Citrate, phosphate, borate and urea were obtained from Sigma (St. Louis, MO, USA). Buffers for high-performance capillary electrophoresis (HPCE) were prepared in MilliQ water and were passed through a 0.22-μm pore size membrane filter (Millex, Millipore, France). To enhance the solubility of CD in the buffers, urea was added to the medium at a concentration of 8 *M*.

2.3. Procedures

2.3.1. Binding constant determination

Salbutamol was dissolved at a concentration of 0.005 g/l in water and was analyzed twice using increasing concentrations of CD in the running buffer. For pH 5 and 7, a citrate phosphate buffer (20 and 40 m*M*, respectively) was prepared. For pH 10.1 and 11, a borate buffer (100 m*M*) was used. For all buffers, the pH was adjusted with 1.0 *M* sodium hydroxide. Determinations of the *K*-values was achieved by calculation of the μ_{ep} of salbutamol and the values were corrected to compensate for the change in buffer viscosity of the electrolyte solutions containing increasing concentrations of either β-CD or Et-β-CD. The range of CD concentrations investigated was from 0 to 200 m*M* except for 6-Et-β-CD (0–50 m*M*) which has a lower solubility in the running buffer. The corrected μ_{ep} ($\mu_{ep,corr.}$) values obtained were plotted against the CD concentration, and these data were analyzed by non-linear regression to assess the agreement with the theoretical model and to determine values for μ_0, μ_c and *K*.

2.3.2. Calculation of the CD solubility in water

The CD solubility studies were performed using the following procedure. First, an excess of the appropriate solid CD was dissolved in water, placed in screw-top vials and mixed in a water bath at 25 or 37°C for 72 h. The excess CD solid-phase was then separated from the solution by filtration through a 0.45-μm pore size membrane. The previously weighed filter was placed in an oven at 110°C until dryness (typically 6 h). The mass of solubilized CD was determined by comparing the mass of the filter and the mass of CD initially introduced in the water. These solubility studies were carried out in triplicate.

2.3.3. Viscosity measurements

Viscosity measurements were carried out with an Ubbelohde micro-viscometer AVS 400 with suspended level bulb (Schott Geräte, Germany). After filling the capillary with about 2.5 ml of the solutions containing CD, the viscometer with its stand was placed in a constant-temperature bath (Schott Geräte) at 25 or 37°C. For each determination, four measurements were carried out, and, after each determination, the micro-viscometer was rinsed with acetone. The corrected efflux time (*t* in s) measured was then multiplied by the constant *k*, to give the kinematic viscosity (η) expressed in mm^2/s: $\eta = kt$, with $k = 0.009874\ mm^2/s^2$.

3. Results and discussion

3.1. Theory

Inclusion complexes are molecular compounds with characteristic structural arrangements, in

which one compound (the host molecule) spatially encloses another (the guest molecule) or at least part of it.

The formation of an inclusion compound in liquid medium is governed by an equilibrium reaction between the guest molecule, salbutamol (S), and the cyclodextrin (CD) host molecule:

$$S + CD \overset{K}{\rightleftharpoons} S\text{–}CD$$

where S–CD is the super molecule of the inclusion compound and K the binding constant.

CDs are non-ionic compounds which do not appreciably absorb UV above 200 nm or visible light. These characteristics are extremely useful in capillary zone electrophoresis (CZE), where they migrate at the velocity of the electroosmotic flow. When a charged solute is included in the cavity of a CD, the inclusion complex has a charge identical to that of the free solute but an increased molecular mass [22]. Since the mass-to-charge ratio of the complex is greater than that of the free solute, the mobility of the solute–CD complex is lower than that of the free solute.

The calculation of stability constants by capillary electrophoresis is based on the following observation: the electrophoretic mobility of a compound S (μ_{ep}) is a function of the proportion of the time that this compound is free and the proportion of the time that it is complexed [15]:

$$\mu_{ep} = \frac{[S]}{[S]+[S\text{–}CD]}\mu_0 + \frac{[S\text{–}CD]}{[S]+[S\text{–}CD]}\mu_c \quad (1)$$

where μ_0 is the electrophoretic mobility of the free salbutamol and μ_c the electrophoretic mobility of the salbutamol–CD complex, [S] and [S–CD] are the concentrations of the free drug and of the inclusion complex, respectively.

Given that

$$[S\text{–}CD] = K[S][CD] \quad (2)$$

Eq. 1 can be rearranged as

$$\mu_{ep} = \frac{\mu_0 + \mu_c K[CD]}{1 + K[CD]} \quad (3)$$

where [CD] represents the concentration of CD in the buffer solution.

The electrophoretic mobility of the salbutamol is readily measured from an electropherogram as

$$\mu_{app} = \mu_{ep} + \mu_{eo} \quad (4)$$

and

$$\mu_{app} = \frac{Ll}{Vt_M} \quad (5)$$

where μ_{app} is the apparent mobility of the salbutamol, L and l the total and the effective length of the capillary, respectively, V the applied voltage and t_M the migration time of salbutamol. Further, μ_{eo} is the mobility of the electroosmotic flow, which is calculated from the t_M of a neutral compound (t_{eo}). Experimentally, μ_{eo} is determined using the t_M of the peak corresponding to water and the following equation:

$$\mu_{eo} = \frac{Ll}{Vt_{eo}} \quad (6)$$

determination of the K-values was achieved by the calculation of μ_{ep} of salbutamol in buffers containing increasing concentrations of either β-CD or Et-β-CD (0–200 mM). These data were analyzed by non-linear regression to assess the agreement with the theoretical model represented by Eq. 3 and to determine values for μ_0, μ_c and K.

Urea, an achiral planar molecule, which itself is soluble up to 16.65 M in water, is well-known for its ability to improve water solubility of various non-polar organic solutes [31]. The addition of urea to the running buffer is expected to decrease the affinity of salbutamol for CD, producing lower binding constants. However, since our purpose was to compare the behaviour of salbutamol with respect to different kinds of CDs, and especially of Et-CDs, and since the addition of urea promotes the solubility of all the CDs investigated, we have assumed that its addition affects all the CDs and thereby all the K-values determined in the same way.

3.2. Correction for viscosity

A first set of experiments was carried out using β-CD added to a borate buffer, pH 10.1, at 37°C and using a constant voltage of 15 kV.

With increasing CD concentration, we observed that both the magnitude of the electrophoretic mobility of salbutamol toward the anodic end and the electroosmotic mobility toward the cathodic end decreased. These results indicate that the solute forms a complex with the CD, and the corresponding increase in its size contributes to a decrease in its electrophoretic velocity. However, the observed decrease in both the electrophoretic and EOF velocities upon CD addition could also be ascribed, at least in part, to an increase in the solution viscosity caused by the addition of CD. To verify this hypothesis, we first measured the viscosity of the various CD solutions employed in the running buffer using a viscometer, and plotted the values obtained against the CD concentration (Fig. 2). A polynomial relationship ($y = 2 \times 10^{-5}x^2 + 0.0009x + 1.0016$, $r^2 = 0.995$) was found between the two parameters.

The μ_{eo} is also related to the viscosity by the following equation:

$$\mu_{eo} = \frac{\zeta\epsilon}{4\pi\eta} \tag{7}$$

where ϵ is the dielectric constant of the medium,

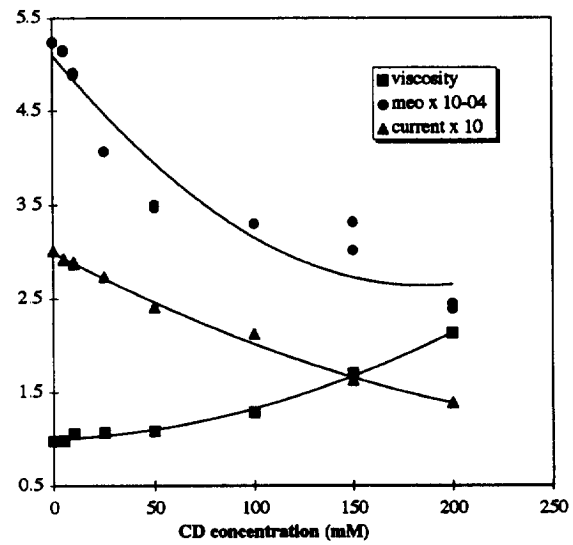

Fig. 2. Changes in the electroosmotic mobility ($cm^2\ s^{-1}\ V^{-1}$), viscosity (η) and current (10 μA) on increasing the β-CD concentration. Conditions: 100 m*M* borate buffer, pH 10.1, with urea 8 *M*, 37°C, 15 kV.

ζ the zeta potential and η the viscosity of the solution.

On the other hand, the addition of a non-ionic compound such as a CD to the electrolyte solution is expected to be responsible for a decrease in the solution conductivity and thereby to a decrease in the generated current (i) [32]. As both μ_{eo} and i are dependent on the viscosity of the running buffer, we also plotted these two parameters as a function of CD concentration (Fig. 2). As expected, a strong dependency was observed between these parameters and CD concentration, indicating that a correction of the μ_{ep} values had to be applied to obtain reliable data. Several approaches to compensate for the changes in the viscosity of the buffer on CD addition were examined [15,24,26,32] and these relied in many cases on the determination of corrected μ_{ep} as follows:

$$\mu_{ep,corr.} = \mu_{ep}\frac{\eta_0}{\eta} \tag{8}$$

where η and η_0 represent the measured viscosity of the buffer in the presence and in the absence, respectively, of CD.

However, one of the major drawbacks of this method is that it requires viscosity measurements for all the electrolyte solutions investigated. This is why we have investigated what would be, between the current (i) and μ_{eo}, the most suitable correction factor readily accessible from one analysis. To this end we calculated the ratios corresponding to $i/i_{(0)}$ and $\mu_{eo}/\mu_{eo(0)}$ obtained at each CD concentration (i and μ_{eo} being the values obtained with a given concentration of CD, up to $i_{(0)}$ and $\mu_{eo(0)}$, the values at zero CD concentration) and plotted them against the η/η_0 ratios. Fig. 3 clearly shows that a linear relationship was observed between $i/i_{(0)}$ and η_0/η ($y = 0.9808x + 0.0363$, $r^2 = 0.9866$). The relationship between $\mu_{eo}/\mu_{eo(0)}$ and η_0/η was not linear, indicating that factors other than viscosity might have affected the μ_{eo} values. In view of these data, $i/i_{(0)}$, being proportional to η_0/η, appeared to be the most consistent factor for correction. However, to validate the correction of μ_{ep} using the $i/i_{(0)}$ ratio, we compared the affinity curves obtained with the initial values of μ_{ep}, μ_{ep}

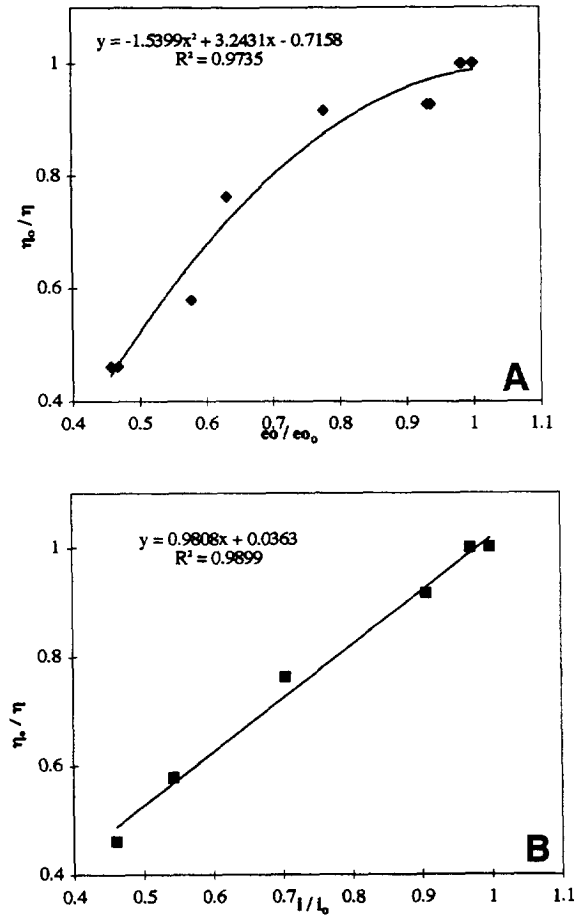

Fig. 3. Relative increase in viscosity against decrease of both electroosmotic mobility (A) and the current (B), measured over a range of β-CD concentrations from 0 to 200 mM. Conditions as in Fig. 2.

corrected by the measured η_0/η ratios, and finally μ_{ep} corrected using the estimated η/η_0 ratio and the following equation:

$$\frac{\eta_0}{\eta} = 0.9808\frac{i}{i_{(0)}} + 0.0363 \quad (9)$$

Fig. 4 shows the excellent fit between the binding curves obtained using μ_{ep} corrected by η_0/η and μ_{ep} corrected by η_0/η estimated using Eq. 9. In addition, the r^2 values indicate that a better correlation with the theoretical model described by Eq. 3 was obtained using a correction of μ_{ep}. This latter result can be explained by the fact that a correction of the μ_{ep} values for changes in

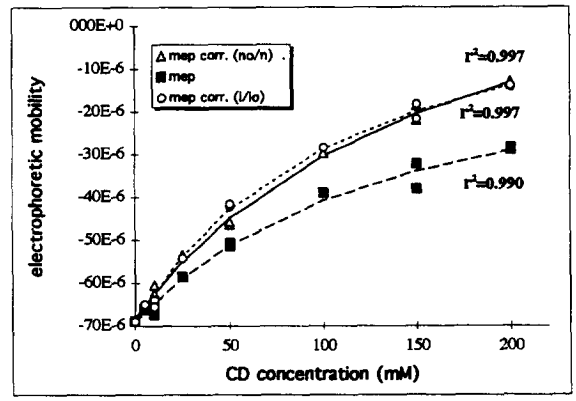

Fig. 4. Binding constant curves obtained with and without correction for viscosity as a function of β-CD concentration. Conditions as in Fig. 2.

viscosity reduces the overall sum of the squares. From these results, we concluded that, using the theoretical model described, the current could be employed as a reliable factor to obtain accurate values of K. Nevertheless, it should be noted that, in spite of the correction employed, standard deviations for K were quite high (R.S.D. ranged from 11 to 18%).

3.3. Effect of pH, voltage and temperature on the binding constant

The preliminary results obtained at pH 10.1 ($K = 9.0\ M^{-1}$) and using the approach described above suggested a weak affinity of salbutamol for β-CD. Rogan et al. [26] reported similar values for the binding constants between the same drug and dimethyl-β-CD. However, previous works on chiral separation using CD by capillary electrophoresis have highlighted the strong dependency of molecule inclusion efficiency on several parameters such as temperature, applied voltage and buffer pH [15–18,21,29,33,34]. In an attempt to examine appropriate conditions for binding constant determination, we subsequently compared the binding results obtained under different operating conditions.

The first parameter we studied was the pH of the running buffer. To determine binding constants, the inclusion complex has to differ significantly in mobility from free salbutamol. This is why experiments have to be carried out under

conditions in which salbutamol is ionized. To this end, several pH conditions were tested: two citrate phosphate buffers, pH 5.0 and 7.0, and two borate buffers, pH 10.1 and 11. Fig. 5 shows the electropherograms obtained with a 100 m*M*

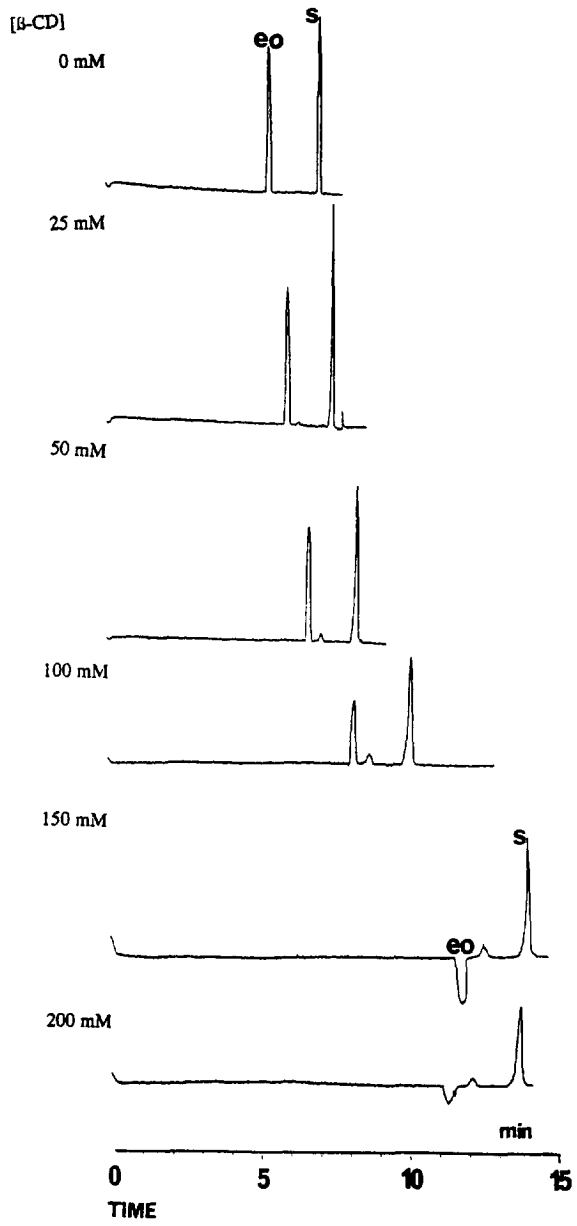

Fig. 5. Electropherograms of salbutamol with increasing concentrations of β-CD (0 to 200 m*M*). Conditions: 100 m*M* borate buffer, pH 11, 37°C and 20 kV.

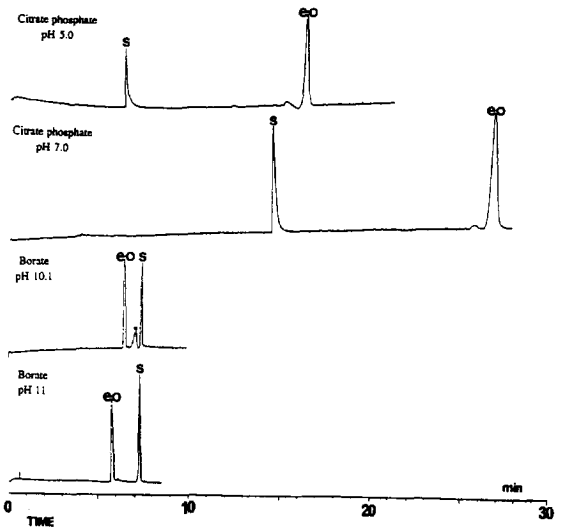

Fig. 6. Electropherograms of salbutamol at various pHs of running buffer, with a 10 m*M* concentration of β-CD. Conditions: 37°C, 30 kV (pH 5), 15 kV (pH 7), 15 kV (pH 10.1) and 20 kV (pH 11).

borate buffer pH 11, with increasing CD concentrations. Fig. 6 displays the comparison of the electropherograms obtained at different pH and using a 10 m*M* concentration of β-CD added to the buffers. When pH values between 7.5 and 9.5 are employed at 37°C, the salbutamol charge is too low, and it migrates with a velocity close to that of the EOF (not shown). Below pH 7.5, salbutamol is positively charged, and its electrophoretic mobility towards the cathode decreases slightly on going from pH 5.0 to 7.0. At pH > 9.5 salbutamol is an anion, and its electrophoretic mobility towards the anode increases with the pH, leading to higher migration times.

For the ionizable salbutamol, we expected the values of the binding constants to vary with the pH. However, results presented in Fig. 7B indicate little or no differences in the *K*-values obtained at pH 7, 10 or 11. The pH was shown to have little influence on the binding constant. According to Wren and Rowe [15], a stronger complexation of an uncharged form of the analyte is expected. This feature would have implied a higher *K*-value at pH 10.1, where salbutamol is still negatively charged but to a lesser extent than at the other pH investigated (the pK_a of the

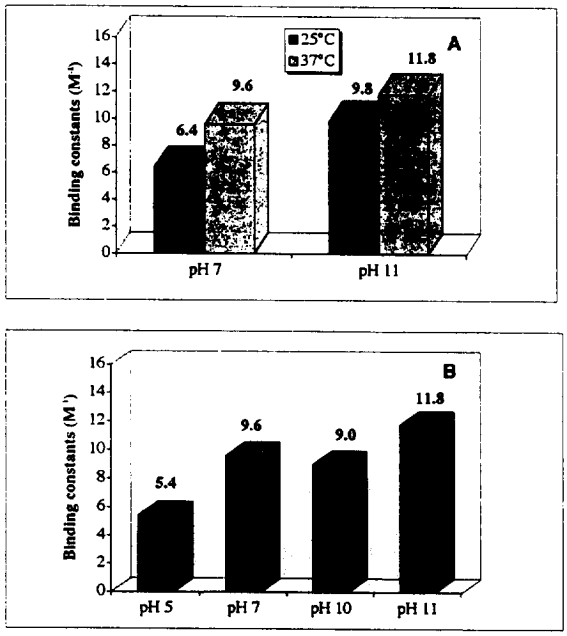

Fig. 7. Influence of the temperature (A) and of the pH (B) on the K values. Conditions: citrate phosphate buffers for pH 5 (20 mM) and 7 (40 mM), respectively, 100 mM borate buffer for pH 10.1 and pH 11.

amino group at 37°C was estimated to be 9.5). These observations therefore reflect the weakness of the affinity of salbutamol for β-CD, becoming independent of parameters which should normally affect it. In spite of a higher migration time, which is supposed to favour the inclusion of salbutamol in the CD, a slightly lower value of the binding constant was observed at pH 5.0. We ascribed this lower affinity of salbutamol to CD at this pH to adsorption of the positively charged salbutamol onto the inner capillary wall. This was indeed confirmed by the peak distortion and the tailing observed.

The binding constants were then determined using three different applied voltages. A difference greater than the S.D. was not found between the values of K obtained for the three voltage values investigated. We concluded that the applied voltage had little influence on the binding constants.

Finally, we observed, as a general trend, that the K-values were slightly higher when the temperature was increased from 25 to 37°C (Fig. 7A), no matter the pH employed. These results are in conflict with those previously reported by others [16,35], who observed a marked decrease in the value of K with an increase in temperature, using other kinds of CDs. Our results may be related to specific characteristics of β-CD, such as the temperature-dependent solubility, which has been estimated at 19 and 23 g/l at 25 and 37°C, respectively.

3.4. Comparison between unmodified β-CD and Et-β-CD

β-CD possesses 21 hydroxyl groups, and derivatized forms are therefore a mixture of products with varying degrees of substitution (DS). Within a CD ring, the DS expressed as the average number of substituted hydroxyls per glucose unit can be any number between 0 and 3.

In view of the conclusions reached with β-CD in the first part of this study, and in order to compare the affinity of salbutamol towards β-CD and Et-β-CD, we then compared the binding constants of these two CDs for salbutamol at 37°C using a citrate–phosphate buffer pH 7. Under these experimental conditions, the chemically modified CD proved to be a more powerful host for the inclusion of salbutamol than β-CD (the K-values were 9.6 and 153 M^{-1}, respectively, for β-CD and Et-β-CD). Although it is still difficult to understand the role of hydroxyl derivatization of CDs in the molecular interaction of the host–guest complex formed, a growing number of studies has served to define some of the factors that influence the affinity of a drug for a given type of CD. Thus, it is generally accepted that inclusion of a molecule in the CD cavity involves hydrophobic interactions, whereas the remainder of the binding interaction and the eventual enantioselectivity are caused by secondary interactions between the chiral centre of the included molecule and the groups located on the CD rim. Hence, it is likely that the alkylation of the hydroxyl groups of β-CD results in a stronger hydrophobic interaction between sal-

butamol and Et-β-CD, ultimately resulting in the higher binding constant obtained for this complex. Moreover, experiments on the influence of the pH have helped to clarify the effect of ethylation on the inclusion of salbutamol in the modified CD. Indeed, in contrast to the observations we made with β-CD, the affinity of salbutamol toward Et-β-CD was more pronounced at pH 7 ($K = 153\ M^{-1}$), where the drug is positively charged, than at pH 11 ($K = 44\ M^{-1}$), where the phenolic group is ionized. This result supports the assumption that the inclusion of salbutamol within the cavity of Et-β-CD has occurred through the aromatic portion of the molecule rather than by the terbutyl extremity. This hypothesis is in agreement with previous workers' observations [34,36]. In particular, St Pierre and Sentell [34] suggested that the inclusion of terbutaline (a molecule similar to salbutamol) in the cavity of β-CD most likely occurred through the aromatic portion of the molecule. However, further studies using NMR spectroscopy are currently under way to add support to this idea. Moreover, at pH 7 the phenolic group of salbutamol is protonated, a situation which is supposed to favour the inclusion of the aromatic portion of the molecule within the CD cavity. In contrast, the charged amino group on the other side of the molecule will affect the entrance of the molecule very little. On the contrary, the charged phenolic group at pH 11 will presumably reduce the host–guest interaction. Seemingly, the presence of the ethylated groups at the CD rim has a stabilizing effect on the interaction, owing to hydrophobic interactions between the ethyl groups and the *tert.*-butyl group on the amine side-chain of the solute. In addition, modification of the CD rim is supposed to modify the type of secondary interaction (e.g. hydrogen bonding) between the CD and salbutamol affinity, and thereby the enantioselectivity of the guest–host complex formation. Under selected conditions, we observed with both β-CD and Et-β-CD a low resolution of the enantiomers, which provides further evidence that the complex formed exists rather as an inclusion of salbutamol in the cavity than as a surface interaction.

3.5. Comparison between 6-Et-β-CD and 10-Et-β-CD

One of the major problems encountered with CD derivatives studies is to ensure a consistency from batch-to-batch of the commercially available CD. It is of particular importance to know the degree of substitution, purity, position of the alkylated groups and alkylation yield in modified CD to obtain reliable data. The main characteristics of the Et-β-CD studied were determined by the manufacturer and are summarized in Table 1. These data show that the two types of Et-β-CD (6- and 10-Et-β-CD), obtained from slightly different synthetic processes, displayed differences which have been evidenced through electrospray mass spectrometry measurements (unpublished data). Moreover, we previously observed a significantly slower release of salbutamol from the 10-Et-β-CD–salbutamol complex, compared with the complex formed with 6-Et-β-CD (Table 1) [13]. Hence, in the last part of our work, our purpose was to establish if the binding constants were correlated to the behaviour observed in the release rate values. Furthermore, to compare the two Et-β-CDs, we chose to work at pH 7, which is near the physiological pH.

However, even in the presence of urea, 10-Et-β-CD could hardly be solubilized in the running buffer at concentrations higher than 10 to 15 m*M*. This limited solubility precludes binding constant determination using the method described above. This is why only qualitative data could be obtained to compare the affinity of salbutamol toward these two ethylated CD. Measurements of corrected μ_{ep} were still possible at 0 and 5 m*M* CD concentrations. We assumed that

Table 1
Principal characteristics of the cyclodextrins studied

	β-CD	6-Et-β-CD	10-Et-β-CD
Degree of substitution	–	1.98	2.12
Molecular mass	1135	1523	1577
Solubility (37°C) (g/l)	23	34	17
Released fraction after 8 h	100%	60%	20%

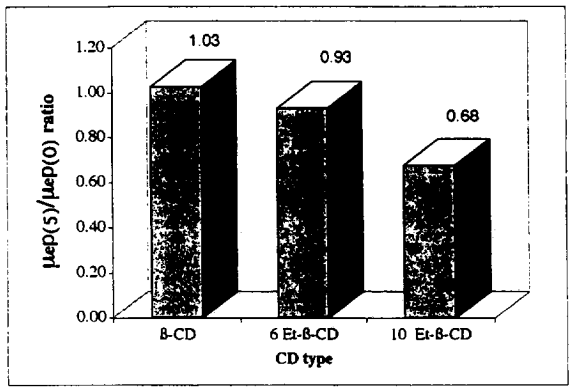

Fig. 8. Comparison of $\mu_{ep}(5)/\mu_{ep}(0)$ ratios obtained for β-CD and the two Et-β-CDs. Conditions: 40 m*M* citrate phosphate buffer, pH 7, urea 8 *M*, 25°C, 15 kV.

the ratio μ_{ep} (5 m*M*)/μ_{ep} (0 m*M*) could reflect the extent of the affinity between salbutamol and the CD. Consequently, the magnitude of the salbutamol binding constant with a given CD could be evaluated, since it was expected to be proportional to this ratio. Accordingly, the lower the value of this ratio, the greater is the affinity. We therefore compared the ratio of μ_{ep} (5 m*M*)/μ_{ep} (0 m*M*) obtained for β-CD, 6-Et-β-CD and 10-Et-β-CD. The results obtained are presented in Fig. 8. As expected, the ratio was lower for 10-Et-β-CD (0.68) compared with the other two CDs investigated. We concluded that affinity of salbutamol to CD estimated by a μ_{ep} (5 m*M*)/μ_{ep} (0 m*M*) ratio represents a good prediction of the release behaviour of drug in the presence of CD.

4. Conclusion

We stress the ability of ACE to obtain accurate values for the binding constants *K* between salbutamol and β-CD or Et-β-CD when using the corrected electrophoretic mobilities to correct for viscosity changes. In this study, the current was demonstrated to be linearly correlated to the viscosity and to represent a valuable factor of correction. We demonstrated that salbutamol has a weak affinity for β-CD, and that its interaction with CD could be greatly enhanced by the ethylation of the hydroxyl groups of the CD. These binding models have been shown to be in good agreement with the salbutamol profile of release observed in the presence of CD and described in a previous work [13].

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