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Use of cyclodextrins in capillary zone electrophoresis

Resolution of terbutaline and propranolol enantiomers

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ABSTRACT

   Terbutaline and propranolol were resolved using capillary zone electrophoresis. The effect of the type and the amount of cyclodextrins added to the background electrolyte on the migration time and the resolution of their enantiomers was studied. Good resolution of the racemic terbutaline was obtained using phosphate buffer at pH 2.5 containing either 5 mM heptakis(2,6-di-O-methyl)-β-cyclodextrin or 15 mM β-cyclodextrin. The background electrolyte, 50 mM phosphate buffer (pH = 2.5) – 4 M urea – 40 mM β-cyclodextrin in 30% (v/v) methanol, on the other hand, gave the best resolution of propranolol enantiomers.

INTRODUCTION

   Cyclodextrins (CDs) are oligosaccharides containing several D-(+)-gluco-pyranose units with a shape similar to a truncated cone able to form inclusion complexes. CDs have been used successfully in analytical chemistry for improving the selectivity of the separation of positional and geometrical isomers and enantiomers [1–4].
   Several techniques have employed CDs for the resolution of enantiomers, *e.g.*, thin-layer chromatography, gas chromatography, capillary isotachophoresis, high-performance liquid chromatography (HPLC), isoelectric focusing, electrokinetic chromatography and capillary zone electrophoresis [5–12]. As enantiomers possess the same chemical properties, they are difficult to separate from each other. Their resolution is generally performed by using a chiral environment that interacts with the analytes either strongly (indirect resolution) or weakly (direct resolution) [13]. Capillary zone electrophoresis (CZE) is a relatively new electrophoretic technique with a high resolving power and high sensitivity used for the separation of compounds with different mobilities [14].
   In this work, CZE was used for the resolution of terbutaline by the direct resolution method. The chiral environment consisted of an aqueous background electrolyte (BGE) containing β-cyclodextrin or heptakis(2,6-di-O-methyl)-β-cyclo-dextrin. The effect of the shape and the amount of CD added to the background

electrolyte on the migration time and the resolution of the two enantiomers of terbutaline was studied.

As the above electrolyte system could not resolve propranolol isomers, we searched for another system and found a BGE containing urea, β-cyclodextrin and methanol to be the best for this purpose.

EXPERIMENTAL

Chemicals

Sodium dihydrogenphosphate, phosphoric acid, ammonium acetate, acetic acid and methanol were purchased from Carlo Erba (Milan, Italy), (*S*)-(−)-propranolol hydrochloride, (*R*)-(+)-propranolol hydrochloride, DL-propranolol hydrochloride, terbutaline hemisulphate salt and β-cyclodextrin (β-CD) from Sigma (St. Louis, MO, USA) and α- and γ-cyclodextrin (α- and γ-CD), and urea from Fluka (Buchs, Switzerland). (+)-Terbutaline and (−)-terbutaline were separated by HPLC according to the published method [7], collected and spiked separately with racemic terbutaline for electrophoretic experiments. Heptakis(2,6-di-O-methyl)-β-cyclodextrin (di-OMe-β-CD) and heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (tri-OMe-β-CD) were obtained from Chinoin (Budapest, Hungary).

Apparatus

Electrophoretic experiments were carried out in an HPE 100 apparatus (Bio-Rad Labs., Richmond, CA, USA) equipped with an on-column UV detector operated at 206 nm. The regulated high-voltage power supply, able to deliver up to 12 kV, was used in either a constant-voltage or a constant-current mode.

A coated capillary tube cartridge (20 cm × 0.025 mm I.D.) (Bio-Rad Labs.) was used for the separations. Injection was done by electromigration by applying a constant voltage.

A Model 2210 line recorder (LKB, Bromma, Sweden) was used for recording the electropherograms.

Background electrolyte (BGE)

A stock solution of 0.1 M NaH$_2$PO$_4$ titrated with phosphoric acid to pH 2.5 was prepared and used for electrophoretic experiments in aqueous solution. The CDs were added separately to the stock solution.

A 50-ml volume of aqueous 0.1 M phosphate buffer prepared as described below was mixed with the appropriate amount of methanol and 4 M urea, and water was added to 100 ml, and the appropriate amount of CD was dissolved in the buffer–methanol solution.

RESULTS AND DISCUSSION

The enantiomeric resolution of racemic compounds can be performed by CZE using either an indirect or direct resolution method. In the direct method a chiral environment interacts with the two enantiomers and forms complexes with different stability constants. Complex formation will influence the effective mobilities of the analytes and thus permit their resolution.

Cyclodextrins have been used successfully in electrophoretic techniques in order to improve the selectivity of the separation of enantiomers. The mechanism is based on inclusion complexation between CDs and the analytes. The structure of the studied compounds has a very important role in the resolution. In fact, the chiral centre of its substituent must be at a favourable distance from the rim of CD in order to effect hydrogen bonding with the hydroxyl groups.

In this study we investigated the effect of α-, β- and γ-CD, 2,6-di-OMe-β-CD and 2,3,6-tri-OMe-β-CD on the migration time and resolution of two drugs, terbutaline and propranolol.

Terbutaline [2-*tert.*-butylamino-1-(3,5-dihydroxyphenyl)ethanol] is a sympa-thomimetic drug-selective β_2-receptor agonist used in the treatment of asthma and lung diseases. Propranolol [1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol] is a β-blocker used in the treatment of angina pectoris [15,16]. The differences in the structures of the two compounds are shown in Fig. 1. It is clear that the chiral carbon of propranolol is at a longer distance from the aromatic group than that in terbutaline. Further, the substituents in the aromatic ring are different.

Different CDs were added to the aqueous BGE for the electrophoretic experiments in order to study the inclusion complexes with the analytes. Based on our previous experience with the resolution of sympathomimetic drugs by CZE, a BGE at a pH of 2.5 was selected [12]. At this pH both terbutaline and propranolol migrate as cations.

α-, β- and γ-CD, di-OMe-β-CD and tri-OMe-β-CD were used as complexing agents in the BGE. The resolution (R) of the enantiomers was calculated by using the equation

$$R = \frac{t(+) - t(-)}{w(+) + w(-)} \cdot 2 \tag{1}$$

where t (s) is the migration time, w (s) the width of the peak at the baseline and $(+)$ and $(-)$ represent the dextro- and laevo-rotatory enantiomers, respectively.

The effect of the concentration of di-OMe-β-CD and β-CD on the migration
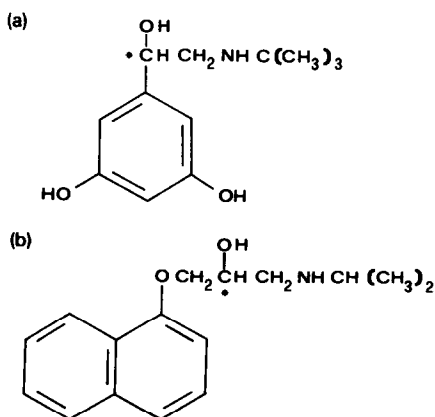
(a)

(b)

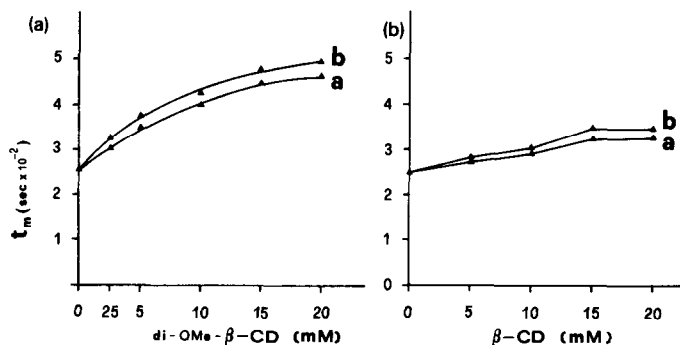Fig. 1. Structures of (a) terbutaline and (b) propranolol.

Fig. 2. Effect of the concentration of cyclodextrins added to the background electrolyte on the migration time of (+)- and (−)-terbutaline. (a) Di-OMe-β-CD; (b) β-CD. BGE: 0.1 M phosphate buffer (pH 2.5); sampling, electrokinetic, 8 kV, 10 s (10^{-5} M terbutaline); electrophoresis, 19 μA (constant), 9 kV.

time of the two enantiomers of terbutaline is illustrated in Fig. 2a and b, respectively. From these results it is clear that by using a higher amount of either di-OMe-β-CD or β-CD the migration time of the analyte compounds is increased, and this is more evident when derivatized CD (di-OMe-β-CD) is used as a chiral selector. This means that di-OMe-β-CD is a better complexing agent than β-CD. This can be explained by considering the structure of terbutaline and the CDs used. Terbutaline has two hydroxyl groups in the aromatic ring that influence the fitting in the cavity of the CD in order to form inclusion complexes. The two methyl groups for each glucose in di-OMe-β-CD influence the hydrophobicity of the CD [4].

In Fig. 3, the resolution of the terbutaline enantiomers is plotted against the concentration of β-CD and di-OMe-β-CD added to the aqueous BGE. In the two chiral environments used the resolution increases by adding a larger amount of CD and the maximum is obtained at 15 mM β-CD and 5 mM di-OMe-β-CD.

The electropherograms for the resolution of terbutaline into its enantiomers by using an aqueous BGE containing 5 mM di-OMe-β-CD ($R = 2.8$) and 15 mM β-CD ($R = 2$) are reported in Figs. 4 and 5, respectively. Both resolutions are very good and the differences consisted in a higher efficiency but lower resolution when β-CD was used. In all the separations performed by CZE, (+)-terbutaline moves towards the
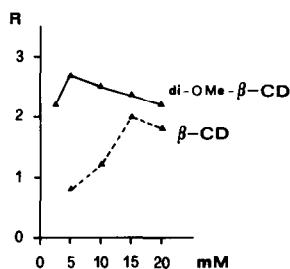

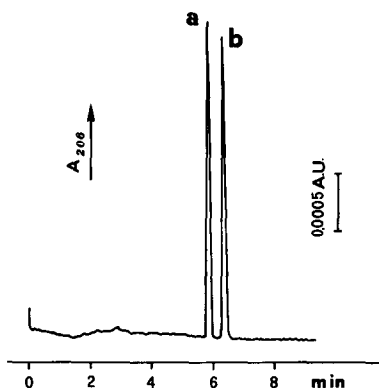Fig. 3. Effect of the amount of cyclodextrins added to the BGE on the resolution (R) of terbutaline enantiomers.

Fig. 4. Electropherogram of the enantiomeric resolution of terbutaline. BGE: 0.1 M NaH$_2$PO$_4$–H$_3$PO$_4$ (pH 2.5) containing 5 mM di-OMe-β-CD. Sampling and electrophoresis as in Fig. 2. (a) (−)-Terbutaline; (b) (+)-terbutaline.

cathode with a lower velocity than the (−)-isomer. This was verified by injecting separately the two enantiomers resolved by HPLC.

Experiments performed by adding different amounts of α- and γ-CD showed no resolution of the two enantiomers. The migration time of the racemic terbutaline was slightly changed. This can be explained by considering the shape of the cavity of the two CDs, which are too small and too large, respectively, for inclusion complex formation with terbutaline.

When using tri-OMe-β-CD a very poor resolution of racemic terbutaline was obtained on adding 80 mM of the modified cyclodextrin to the aqueous BGE. On
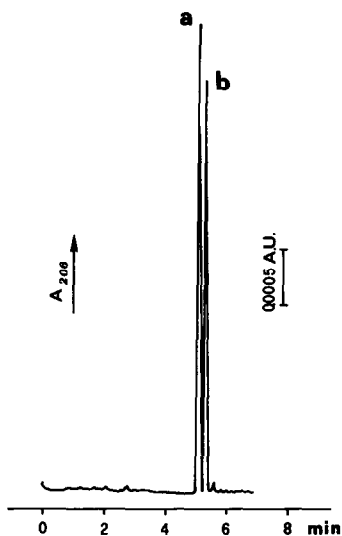

Fig. 5. Electropherogram of the separation of racemic terbutaline: (a) (−)-terbutaline; (b) (+)-terbutaline. BGE: 0.1 M phosphate buffer (pH 2.5) containing 15 mM β-CD. Sampling and electrophoresis as in Fig. 2.

increasing the amount of triOMe-β-CD the migration time changed (from 250 s at 0 mM to 277 s at 80 mM). This shows that the inclusion complex are probably formed but this modified CD gives a poor enantioselectivity effect. Of course, the enantioselectivity is improved when hydroxyl groups are on the rim of the CD and interact with the analytes by hydrogen bonding.

The same BGE used for the resolution of racemic terbutaline was tested for the separation of the enantiomers of propranolol. Different CDs (α-, β- and γ-CD, di-OMe-β-CD and tri-OMe-β-CD) were added separately to the aqueous BGE for the electrophoretic experiments and the migration time was measured. α-CD did not markedly influence the migration time of propranolol, consequently giving no resolution of the two enantiomers. The use of γ-CD, β-CD, di-OMe-β-CD and tri-OMe-β-CD affected the retardation of propranolol. In fact, its velocity diminished on increasing the amount of CD added to the BGE, showing an inclusion complexation of propranolol with these type of CDs. The results are reported in Fig. 6.
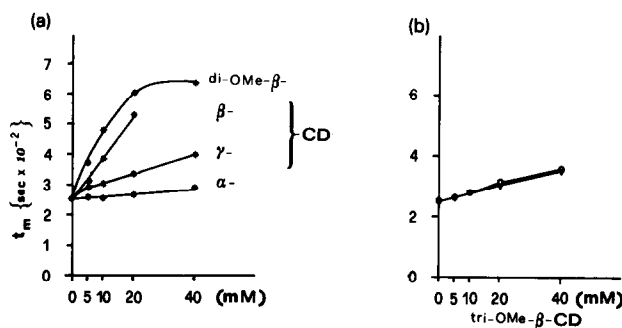

Fig. 6. Effect of the amount of cyclodextrins added to the BGE on the migration time of propranolol. BGE: 0.1 M phosphate buffer (pH 2.5) containing the appropriate amount of CD. Sampling: electrokinetic, 8 kV, 10 s (10^{-5} M racemic propranolol); electrophoresis as in Fig. 2. (a) α-, β-, γ-CD and di-OMe-β-CD; all curves racemic propranolol. (b) Tri-OMe-β-CD; ($\bigcirc$) (R)-(+)-propranolol; ($\blacktriangledown$) (S)-(−)-propranolol.

Under the experimental conditions used (pH, ionic strength, chiral selector, solvent, etc.), no resolution was achieved for the two enantiomers of propranolol except when tri-OMe-β-CD was used. The best resolution of the two enantiomers was achieved by adding 40 mM of the CD derivative to the BGE (R < 0.5). Armstrong et al. [17] reported the resolution of propranolol enantiomers by HPLC using two 25-cm β-cyclodextrin columns in series. They also showed the structure of the inclusion complexes of the two enantiomers with β-CD. Thus, considering the success achieved by HPLC and the theoretical considerations about the inclusion complexes formed, we tried increasing the amount of the chiral selector in the aqueous BGE. As previously reported, aqueous solutions of urea are able to solubilize appreciable amount of β-CD and, further, urea does not bind CDs [18,19].

Experiments carried out by using 50 mM phosphate buffer (pH 2.5) containing 4 M urea and different amounts of β-CD ranging from 30 to 80 mM gave no resolution of racemic propranolol. We therefore tried a BGE with methanol as an organic modifier and containing 4 M urea and 40 mM β-CD. The methanol content in the BGE
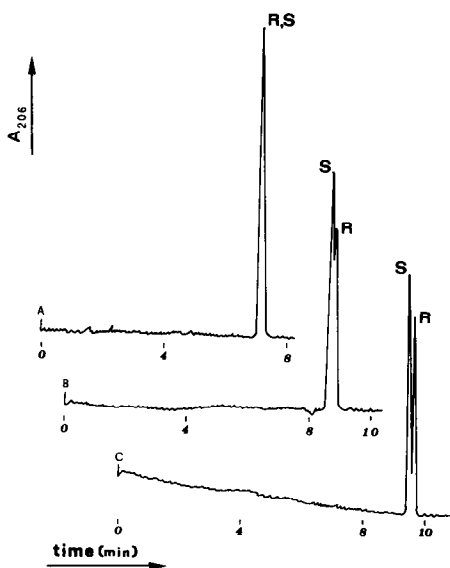
Fig. 7. Electropherograms of racemic propranolol separation. BGE: 50 mM phosphate buffer (pH 2.5) containing 4 M urea, 40 mM β-CD and increasing amounts of methanol (A, 0%; B, 10%; and C, 30%). Electrophoresis, 8 kV (constant), 4–6 μA; sampling, 4 kV, 5 s.

ranged from 0 to 40% (v/v). The electrophoretic resolution of (S)-$(-)$- and (R)-$(+)$-propranolol is reported in Fig. 7. It is evident that the resolution of racemic propranolol depends on the amount of organic modifier; the resolution was improved by increasing the methanol content, the best resolution being obtained when the BGE contained 30% of methanol. Although the mechanism of the separation is not clear, the most important effect is probably due to the change in the solvation of the included isomers. No further improvement in the resolution of the drug was obtained by increasing the amount of β-CD in this electrolyte system. The use of tri-OMe-β-CD in a phosphate–methanol buffer solution gave no resolution of racemic propranolol.

CONCLUSIONS

The results demonstrate that in CZE cyclodextrins are good enantioselective agents for the resolution of terbutaline. The resolution is obtained in less than 6 min. Terbutaline forms complexes with β-CD and di-OMe-β-CD and tri-OMe-β-CD, and the complexation increases with increase in the amount of the CDs added to the BGE.

The shape of the CD and the guest compound influence the resolution of the enantiomers. The use of urea and methanol influence the selectivity for the resolution of racemic propranolol. Propranolol was resolved into its enantiomers in a relatively short time (less than 10 min).

CZE can provide several advantages over other analytical techniques for enantiomeric resolution, e.g., high resolution high efficiency and short analysis times. Further, in CZE expensive chiral compounds are not needed; in fact CDs are added to

the BGE at a relatively low concentration and only a few millilitres of the BGE are used in the electrophoretic experiments. On the other hand, the use of CZE for preparative purposes needs further work.

The use of a relatively short capillary (20 cm) and a small I.D. (0.025 mm) allow the broadening of the separated zones due either to diffusion and or Joule heating to be minimized. Further, the coating of the capillary eliminates the electroosmotic flow and thus improves the resolution [20].

The detection limit, *i.e.*, the minimum sample concentration that could be injected by the electrophoretic method to obtain a signal-to-noise ratio of 2:1, was found to be 1.10^{-7} M for terbutaline.

Analytical CZE can be used successfully for enantiomeric purity control of drugs (terbutaline and propranolol). Further, pharmacokinetic studies of terbutaline could be performed but the sensitivity should be improved, *e.g.*, by using an electrochemical detector, with CZE coupled with mass spectrometry.

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