# Use of β-cyclodextrin polymer as a chiral selector in capillary electrophoresis

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## Abstract

Enantiomers of several basic compounds of pharmaceutical interest were successfully separated by capillary electrophoresis using a modified β-cyclodextrin polymer. As the cyclodextrin contained carboxylic groups, the chiral selector could be used in either an uncharged or a charged mode, selecting the appropriate pH of the background electrolyte. The effect of the pH of the background electrolyte on the effective mobility, resolution and selectivity was studied in the range 2.6–6.2 for the enantiomer resolution of β-hydroxyphenylethylamine, norphenylephrine, terbutaline, ephedrine, norephedrine, ketamine, epinephrine and propranolol. Very good enantiomeric resolution was achieved for all the compounds except for ephedrine and norephedrine ($R < 0.5$). An increase in the pH of the electrolyte caused an inversion of mobility for either terbutaline and propranolol owing to strong complexation with the negatively charged polymer.

## 1. Introduction

The resolution of enantiomers is a growing field of interest in analytical chemistry, especially for biomedical and pharmaceutical analysis, because often two enantiomers of the same drug show different pharmacological or bioactive effects. Therefore, rapid, sensitive, selective and high-resolution analytical methods are required for, e.g., chiral purity control of drugs, pharmacokinetic studies and drug metabolism analysis [1].

Among the different methods used for this purpose, high-performance liquid chromatography (HPLC) has often been applied in this field using a wide variety of chiral columns in which different resolution mechanisms have been applied [2].

In recent years, capillary electrophoresis (CE) with its high resolving power has attracted great interest for the analysis of different classes of compounds including enantiomers [3–6]. Owing to the similar physico-chemical properties of enantiomers, they cannot be separated by CE unless a chiral environment is used in order to modify selectively their properties and thus the effective mobility. A chiral compound is either added to the background electrolyte [7–12] or bonded to the capillary wall [13] and the method is called the direct resolution method. Here labile diastereomeric complexes are formed during the electrophoretic process and if they exhibit different stability constants the effective mobility is selectively modified and their sepa-

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ration is achieved. So far, chiral surfactants, proteins, chiral metal complexes and cyclodextrins have been used as chiral selectors for enantiomeric resolution by CE [3–6,8–10].

Cyclodextrins (CDs) are chiral compounds with the shape of a truncated cone; they are neutral and natural oligosaccharides that possess a hydrophobic cavity and a hydrophilic exterior. The presence of chiral carbons in their structure allows enantioselective interactions with several analytes. Inclusion complexation between analytes and the CD cavity stabilized by hydrogen bonds with hydroxyl groups of the CD is the main interaction involved in the enantioseparation mechanism [6,14].

Modified CDs have been shown to give a greater possibility of resolution for those compounds which cannot be resolved using the native CD, e.g., sympathomimetic drug enantiomers were completely separated with di-O-methyl-$\beta$-CD [11] and $\alpha$-hydroxy acid enantiomers with methyl amino $\beta$-CD [12].

Another approach for improving the selectivity of enantiomer separation when CDs are used as chiral selector is the addition of non-chiral compounds, e.g. organic solvents [15,16] or urea [16], or the use of polymers in the chiral environment [17].

Gels in capillaries, mainly used for the separation of biopolymers [18], have been employed either as support matrices or as modified gels of CD [19,20].

Being interested in enantiomer resolution by CE and encouraged by recent results using modified CDs [12,21], we extended our study to the use of a $\beta$-CD polymer containing carboxylic groups. The polymer was added to background electrolytes at different pH values for the enantiomer separation of several basic compounds of pharmaceutical interest.

## 2. Experimental

### 2.1. Apparatus

Electrophoretic experiments were performed on a Biofocus 3000 system (Bio-Rad, Hercules, CA, USA) equipped with a UV–visible multiwavelength detector operating either at a single wavelength (206 nm) or in the scanning mode (190–360 nm). Fused-silica capillary tube [40 cm (effective length 35.5 cm) × 50 $\mu$m I.D.] was obtained from Polymicro Technologies (Phoenix, AZ, USA) and a coated capillary cartridge (17 cm × 25 $\mu$m I.D.) from Bio-Rad. The polyimide coating of the former was removed from the capillary to create a window at the appropriate position with several drops of concentrated $H_2SO_4$ at 100°C. The capillary was then positioned in the cartridge. An applied voltage of 15 kV was used and the grounded compartment (close to the detector) was negative. The capillary cartridge was thermostated with circulating liquid at 25°C. The temperature of the carousel compartment was 25°C. Injection of samples was done by the electrokinetic method except for the measurements of the electroosmotic flow, which were done by pressure 5 p.s.i. s, corresponding to $35 \cdot 10^{-3}$ MPa for 1 s.

The new capillary (uncoated) was pressure-rinsed with 0.5 $M$ NaOH for 10 min and then water (30 min) in order to activate the silica on the wall.

The following washing steps were used prior to each electrophoretic run: (1) water for 60 s; (2) 0.010 $M$ NaOH for 60 s; (3) water for 200 s; (4) background electrolyte for 60 s.

### 2.2. Chemicals

Soluble anionic $\beta$-CD polymer (for its characteristics see Table 1) was purchased from Cyclolab (Budapest, Hungary). ($\pm$)-Epinephrine, D,L-$\beta$-hydroxyphenethylamine, D,L-pro-

Table 1
Characteristics of anionic $\beta$-cyclodextrin polymer

| Polymer type | Carboxymethylated-$\beta$-cyclodextrin |
|---|---|
| Producer | Cyclolab (Budapest, Hungary) |
| Molecular mass | 6000–8000 |
| Solubility in water | >20% |
| CD content | 50–60% |
| COO⁻ content | 3–4% |
| COO/CD | 2 |
| Cross-linking | 1-Chloro-2,3-epoxypropane |

pranolol, ketamine, (±)-ephedrine, (±)-ter-butaline, (+)-ephedrine, (−)-ephedrine and (−)-epinephrine were obtained from Sigma (St. Louis, MO, USA) (±)-norphenylephrine from Aldrich (Steinheim, Germany) and methanol, sodium dihydrogenphosphate, sodium acetate, acetic acid and phosphoric acid from Carlo Erba (Milan, Italy).

The background electrolyte (BGE) composition was 0.065 $M$ phosphate buffer (pH 2.5), 0.051 $M$ phosphate buffer (pH 3.5), 0.05 $M$ sodium acetate–acetic acid (pH 4.5) and 0.075 $M$ phosphate buffer (pH 6.2). The buffers of pH 2.5 and 3.5 were prepared by dissolving the appropriate amount of $NaH_2PO_4$ in 50 ml of doubly distilled water, titrating the mixtures with tenfold diluted $H_3PO_3$ (85%) and adjusting the volume to 100 ml, while for the buffer at pH 6.2 $Na_2HPO_4$ was used.

CD polymer was dissolved daily in the buffer and the solutions were filtered through a 0.45-$\mu$m filter.

Stock solutions of $2 \cdot 10^{-3}$ $M$ racemic standard were prepared and stored at 4°C and diluted with doubly distilled water to $2 \cdot 10^{-5}$ $M$ or as otherwise described.

## 3. Results and discussion

One of the main drawbacks encountered when $\beta$-CD is used as a chiral additive in capillary electrophoresis is its low solubility in water (about 1.8%, w/v). One solution to this could be to use a modified $\beta$-CD with a higher solubility than the parent compound. The modification is achieved by changing one or more hydroxyl groups on the rim of the CD by introducing other groups, e.g., sulphate, phosphate, carboxylate, methylamino, by chemical reaction.

As shown previously, the use of modified CDs in CE not only allows the application of larger amounts of CD but can also markedly improve the selectivity of enantiomer separation [11,12,18]. Chemical modification of a CD can change the hydrophobicity of the cavity and can allow the formation of other stereoselective bonds (polar, hydrophobic) between substituent groups on the CD cavity and on the chiral centre of the analytes.

$\beta$-CD polymer was used as a chiral selector additive to the BGE for the enantiomeric separation of several basic compounds. The analysed compounds (for the structures see Fig. 1) were run in a BGE at different pH in the range 2.5–6.2. They moved in the direction of the cathode owing to the protonation of the amino group. The apparent mobility, $\mu_{app} = \mu_e + \mu_{eof}$ (where e and eof represent effective and electroosmotic flow, respectively) was calculated using the following equation:

$$\mu_{app} = \frac{lL}{t_x V} + \frac{lL}{t_0 V} \tag{1}$$

where $l$ and $L$ are the effective and total length of the capillary, respectively, $V$ the applied voltage and $t_x$ and $t_0$ the migration time of the sample and the electroosmotic marker, respectively.

Different amounts of $\beta$-CD polymer (0–20 mg ml$^{-1}$) were added to the BGE in order to study the effect of the concentration of the chiral selector on the electrophoretic mobility and resolution of the analysed compounds.

Fig. 2 illustrates the electrophoretic process at pH 2.5 and 4.5. At a low pH the carboxylic groups of the CD polymer are protonated and therefore the chiral selector is moving with the velocity of the electroosmotic flow as a quasi-stationary phase. An increase in pH will cause the polymer to be negatively charged owing to the dissociation of the carboxylic groups; in this case the velocity of the chiral selector is influenced not only by the charge of the analyte and the electroosmotic flow but also by the electrophoretic mobility of the complex formed between the analyte and the polymer.

### 3.1. Effect of concentration of $\beta$-cyclodextrin polymer and pH on effective mobility

Fig. 3 shows the effect of $\beta$-CD polymer on the effective mobility of the analysed drugs when a BGE at pH 2.5 was used. On increasing the concentration of the chiral selector on the BGE,
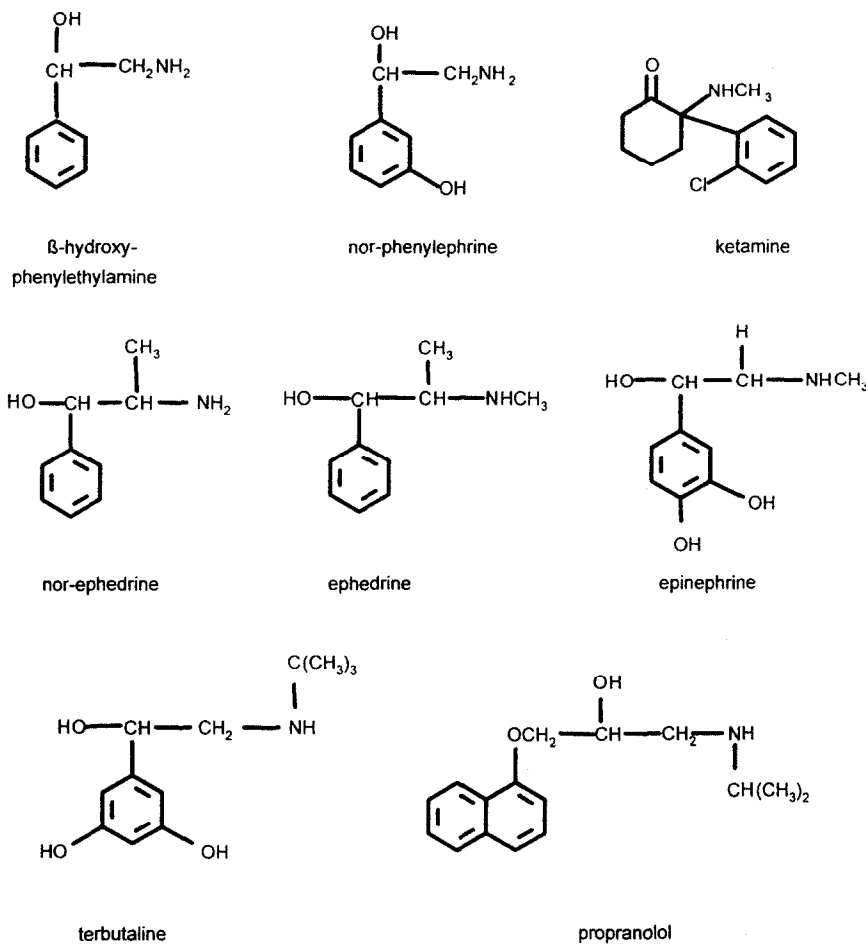
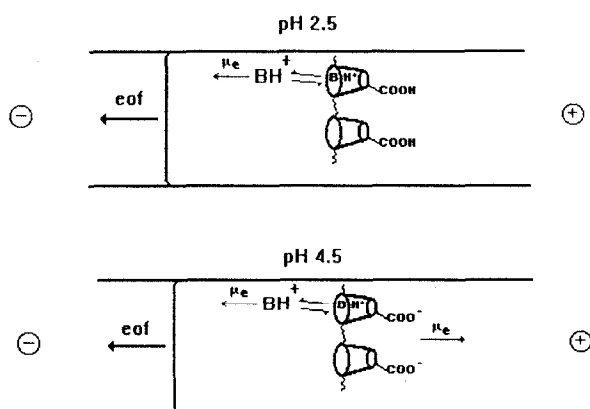Fig. 1. Structures of the standard compounds used in the electrophoretic study.

Fig. 2. Illustration of the electrophoretic process when a chargeable $\beta$-CD polymer is used as a chiral additive to the background electrolyte.

a general decrease in mobility was recorded owing to complexation between the analytes and $\beta$-CD polymer. The affinity of the CD polymer towards the analytes was found to be propranolol > terbutaline > norphenylephrine > $\beta$-hydroxyphenylethylamine = ephedrine = norephedrine > epinephrine, considering the estimated difference of effective mobility $\Delta\mu_e = \mu_{e20} - \mu_{e0}$, where $\mu_{e0}$ and $\mu_{e20}$ are the effective mobilities of the sample in absence and presence of 20 mg ml$^{-1}$ of CD, respectively ($\mu_{e20}$ is referred to the enantiomer with lower mobility). The electroosmotic flow was measured by separately injecting benzyl alcohol and methanol (195 nm) and no noticeable change was found on increasing the concentration of the CD in the BGE
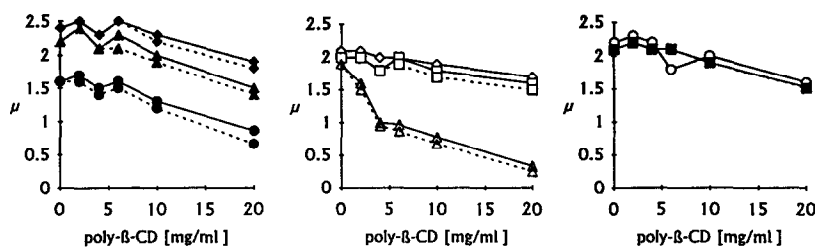
Fig. 3. Effect of $\beta$-CD polymer concentration on the effective mobility of the studied compounds. Run conditions: capillary, 40 cm (35.5 cm to detector) × 0.050 mm I.D., uncoated; background electrolyte, 0.065 $M$ phosphate buffer (pH 2.5) containing the appropriate amount of $\beta$-CD polymer; detection wavelength 206 nm; applied voltage, 15 kV; current, 38 $\mu$A; temperature, 25°C; injection, electrokinetic, 5 kV, 5 s. ◆ = $\beta$-Hydroxyphenylethylamine; ▲ = norphenylephrine; ● = terbutaline; ◇ = ketamine; □ = epinephrine; △ = propranolol; ■ = ephedrine; ○ = norephedrine. $\mu$ in $10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$.

($0.64 \cdot 10^{-4}$ and $0.52 \cdot 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$ at 0 and 20 mg ml$^{-1}$ of CD, respectively).

In order to verify the effect of pH on the effective mobility of the compounds studied, electrophoretic experiments were performed using the BGE at pH 3.5, 4.5 and 6.2. Fig. 4 shows the effect of the pH on the electroosmotic flow, $\mu_{eof}$, with and without 10 mg ml$^{-1}$ of poly-$\beta$-CD. As expected, the electroosmotic flow rose on increasing the pH of the BGE. However,

the addition of the $\beta$-CD polymer to the BGE at the operating pH did not markedly influence the electroosmotic flow.

Fig. 5 shows the influence of the concentration of poly-$\beta$-CD added to the BGE at pH 4.5 on the effective mobility of the compounds studied. When the analysis was performed in the BGE at pH 4.5 in the absence of chiral polymer, the migration time of the analysed compounds decreased with increase in pH owing to the increase in the electroosmotic flow. The addition of the chiral additive to the BGE caused a general decrease in effective mobility owing to the complexation of the analytes with the polymer additive. At pH 4.5 we observed a stronger complexation than that obtained at lower pH, which can be ascribed to the charge of the $\beta$-CD polymer. This effect is markedly observed when 6 mg ml$^{-1}$ of poly-$\beta$-CD was used for propranolol enantiomers that are moving behind the electroosmotic flow. This means that their complexation is so strong that the diastereomeric complexes are negatively charged, but the two
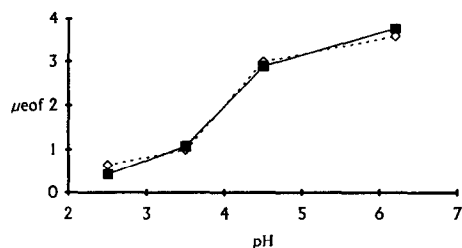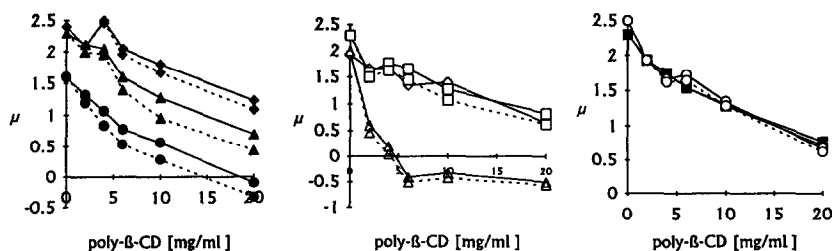


Fig. 4. Effect of pH of the background electrolyte on the electroosmotic flow ($\mu_{eof}$) when the background electrolyte was used in (◇) the absence and (■) the presence of 10 mg ml$^{-1}$ of chiral polymer; $\mu_{eof}$ in $10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$.



Fig. 5. Effect of the concentration of poly-$\beta$-CD on the effective mobility of the studied compounds at pH 4.5. Run conditions: background electrolyte, 0.05 $M$ sodium acetate (pH 4.5); applied voltage, 15 kV; current, 39 $\mu$A; other experimental conditions as in Fig. 3. For symbols, see Fig. 3; $\mu$ in $10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$.

isomers are moving in the direction of the cathode owing to the presence of a relatively strong electroosmotic flow.

The same effect was recorded for terbutaline enantiomers but when 20 mg ml$^{-1}$ of poly-$\beta$-CD were added to the BGE at pH 4.5. A similar effect was recently observed using chargeable cyclodextrins [18].

Inversion of the electrophoretic mobility was also obtained at pH 3.5 when the BGE was supported with 10 mg ml$^{-1}$ of poly-$\beta$-CD but only for propranolol (results not shown).

Fig. 6 shows as an example the separation of a standard mixture containing $\beta$-hydroxy-phenylethylamine, terbutaline and propranolol, where inversion of the mobility of propranolol and terbutaline can be observed.

Electrophoretic runs performed at pH 6.2 with different amounts of chiral polymer showed a general decrease in the electrophoretic mobility for all the compounds analysed that was more evident than at lower pH. We conclude that the affinity of the analysed compounds for poly-$\beta$-CD is as follows [after considering $\Delta\mu_{e(20-)}$]: propranolol > terbutaline > norphenylephrine > norephedrine > $\beta$-hydroxyphenylethylamine > epinephrine > ketamine. It is clear that the

complexation is strongly influenced by the pH of the BGE, increasing concomitantly.

### 3.2. Effect of concentration of $\beta$-cyclodextrin polymer and pH on enantiomer resolution

The resolution, $R$, was calculated using the following equation:

$$R = 2\left(\frac{t_2 - t_1}{w_2 + w_1}\right) \qquad (2)$$

where $t_2$ and $t_1$ are the migration times and $w_2$ and $w_1$ the widths at the baseline of the two enantiomers of lower and higher mobility, respectively.

Fig. 7 shows the effect of the amount of poly-$\beta$-CD added to the BGE at pH 2.5, 3.5, 4.5 and 6.2 on the resolution of the studied racemic mixtures.

At low pH (2.5) the enantiomers were not resolved for either ketamine or norephedrine, even if the concentration of the chiral additive was increased, while ephedrine enantiomers showed poor resolution ($R < 0.5$) at 20 mg ml$^{-1}$ of poly-$\beta$-CD. This was not surprising considering that previously we did not resolve these compounds with $\beta$-CD at pH 2.4 [11].

Considering that inclusion complexation is probably a stereoselective resolution mechanism, we can assume that the aromatic groups of the three analysed compounds fit the cavity of the $\beta$-CD and hydrogen bonds are formed between hydroxyl and nitrogen groups in the chiral centres of ephedrine and norephedrine with hydroxyl and carboxyl groups on the rim of the CD. In the case of ketamine these possibilities are reduced because no hydroxyl substituents are in the chiral centre.

The chemical structure of epinephrine is similar to that of ephedrine, the difference being in the presence of hydroxyl groups at position 3 and 4 of the aromatic ring. When epinephrine was analysed at pH 2.5, poor enantiomer resolution was obtained when the BGE was supplemented with 6 mg ml$^{-1}$ of poly-$\beta$-CD. The resolution was improved by increasing the amount of chiral additive ($R = 1$ and 2.4 at 10 and 20 mg ml$^{-1}$ of
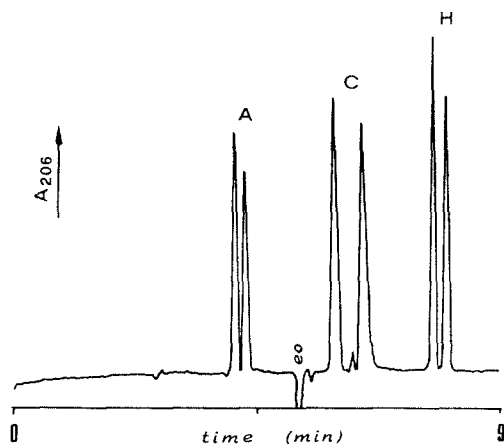


Fig. 6. Electropherogram of the separation of enantiomers of (A) norphenylephrine, (C) terbutaline and (H) propranolol. Background electrolyte, 0.05 $M$ acetate buffer (pH 4.5) and 20 mg ml$^{-1}$ of poly-$\beta$-CD; applied voltage, 15 kV; current, 39 $\mu$A; sampling, 5 kV, 5 s of (A, B) $5 \cdot 10^{-5}$ $M$ and (C) $5 \cdot 10^{-6}$ $M$.
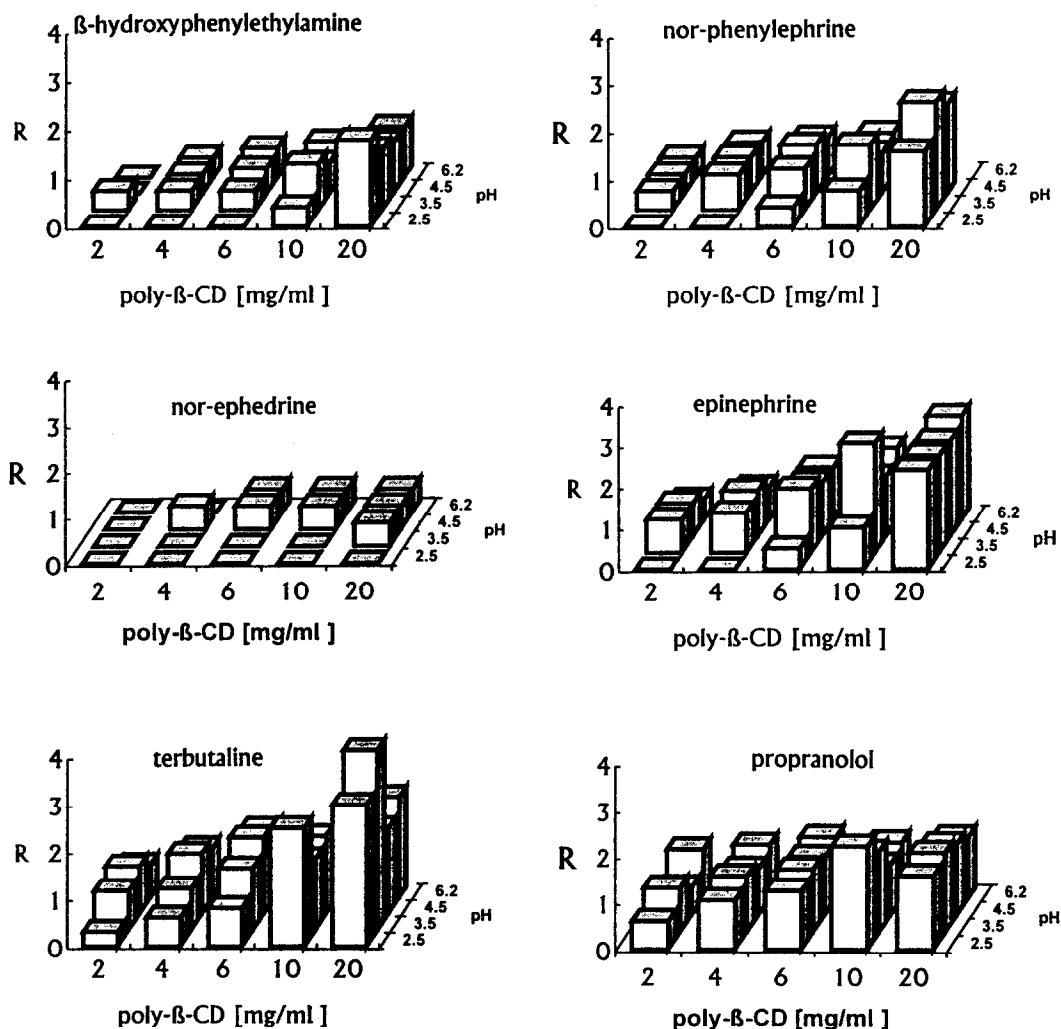
Fig. 7. Effect of the concentration of $\beta$-CD polymer on the resolution of the sample compounds at different pH. Applied voltage, 15 kV; current, 38–62 $\mu$A. For other experimental conditions, see text.

poly-$\beta$-CD, respectively). In order to explain the higher resolution of the two enantiomers of epinephrine compared with the results obtained for ephedrine, we can assume that the two hydroxyl groups on the aromatic ring form stabilizing bonds with the chiral polymer.

In order to obtain a baseline resolution of norphenylephrine and $\beta$-hydroxyphenylethyl-amine, it was necessary to use 6 and 10 mg ml$^{-1}$ of poly-$\beta$-CD at pH 4.5 and 3.5, respectively. Also in this instance the resolution was influenced by the amount of chiral selector added to

the BGE and by the pH. The maximum resolution, $R = 2.25$, was recorded at pH 3.5 for norphenylephrine and $R = 1.77$ at pH 2.5 for $\beta$-hydroxyphenylethylamine when the BGE was supported with 20 mg ml$^{-1}$ of polymer. For both compounds an increase in the pH of the BGE caused a decrease in $R$ when the same amount of chiral additive was used.

The enantiomer resolution of norphenyl-ephrine at pH 6.2 (20 mg ml$^{-1}$ of poly-$\beta$-CD) could not be measured owing to the migration of one of the two resolved enantiomers with the

electroosmotic flow. This is, of course, a limitation for quantitative analysis when charged poly-$\beta$-CD is used as a chiral additive for enantiomer separation. The same drawback was observed for terbutaline and epinephrine at pH 6.2 (10 and 20 mg ml$^{-1}$ of polymer, respectively). This effect is depicted in Fig. 8b while Fig. 8a shows a good separation of (+)- and (−)-epinephrine obtained at the same pH but at a lower concentration of chiral additive.

Terbutaline and propranolol showed maximum resolution at pH 3.5 ($R = 3.8$) and 2.5 ($R = 2.22$) with 20 and 10 mg ml$^{-1}$ of poly-$\beta$-CD, respectively. The two compounds were baseline resolved even when 2 mg ml$^{-1}$ of chiral polymer was added to the BGE at pH 6.2 (propranolol) and 4.5 (terbutaline). At relatively high concentrations of poly-$\beta$-CD (10 and 20 mg ml$^{-1}$) an increase in pH caused a decrease in resolution for terbutaline and propranolol whereas at lower concentration the resolution increased. Also in this instance the amount of polymer had a very important effect on the resolution; in fact, $R_s$ generally increased with increasing amount of chiral additive. The results indicated that the use of a pH lower than 4.5 resulted in a better resolution of both proprano-
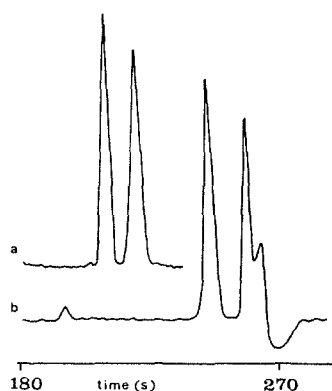
lol and terbutaline enantiomers when the concentration of the chiral selector was 10 and 20 mg ml$^{-1}$. It seems that the strong ion-pairing effect slightly deteriorates the resolution. To explain the stronger complexation of terbutaline and propranolol with the additive in comparison with the other analytes studied we have to consider the inclusion complexation with the $\beta$-CD in the polymer, the ion-pair interaction and probable adsorption.

Further, a general decrease in $R$ was obtained at pH 6.2 for terbutaline and propranolol probably owing to the strong electroosmotic flow that caused a decrease in migration time compared with those obtained at lower pH. The chiral recognition is a result of dynamic equilibrium between the enantiomers and the chiral selector; thus at pH 6.2 the number of dynamic exchanges is smaller owing to the shorter time. Hence we can stress the importance of the control of the electroosmotic flow for the optimum experimental separation conditions when such a polymer is used in CE.

Experiments performed by injecting the enantiomers of propranolol showed that the $R$-isomer forms more stable diastereomeric complexes than the $S$-isomer with the poly-$\beta$-CD; in fact, in all instances the $R$-isomer moved with a longer migration time. As can be seen in Fig. 1, the charged CD polymer moves in the opposite direction to the electroosmotic flow, decreasing the effective mobility of the two isomers. With epinephrine the (+)-isomer moved with a lower effective mobility than its enantiomer, showing a higher affinity for the chiral selector.

Experiments performed in a coated capillary (17 cm × 0.025 mm I.D.) allowed rapid enantiomeric resolution even with a relatively small amount of chiral polymer. At pH 2.5 no noticeable electroosmotic flow was recorded but very broad peaks were obtained for propranolol and terbutaline enantiomers, probably owing to adsorption of the analytes. The negative effect was more evident when the amount of chiral selector was increased. An increase in the pH of the BGE caused a strong electroosmotic flow and poor reproducibility of the migration time, probably owing to adsorption of poly-$\beta$-CD on the



Fig. 8. Electrophoretic separation of a racemic mixture of epinephrine at pH 6.2. The background electrolyte contained (a) 10 and (b) 20 mg ml$^{-1}$ of poly-$\beta$-CD. Run conditions: applied voltage, 15 kV; current, 60 $\mu$A; injection, 7 kV, 7 s of $5 \cdot 10^{-5}$ M racemic epinephrine; capillary, 40 cm × 0.05 mm I.D. (uncoated).

capillary wall, which will be negatively charged. Unfortunately, the nature of the coating of the capillary is not known (this is a Bio-Rad patent), so it is not possible to understand the interactions between the polymer used as the chiral selector and the capillary wall.

Fig. 9a shows, as an example, the electrophoretic separation of a standard mixture containing (R) and (S)-propanolol (enriched in the R-isomer) using a coated capillary. By reversing the polarity (the analytes were moving as anions towards the anode) we obtained inversion of the migration order of the two enantiomers (see Fig. 9b).

So far the use of coated capillaries has been hindered by the irreproducibility of the analytical results obtained. We have been trying to improve
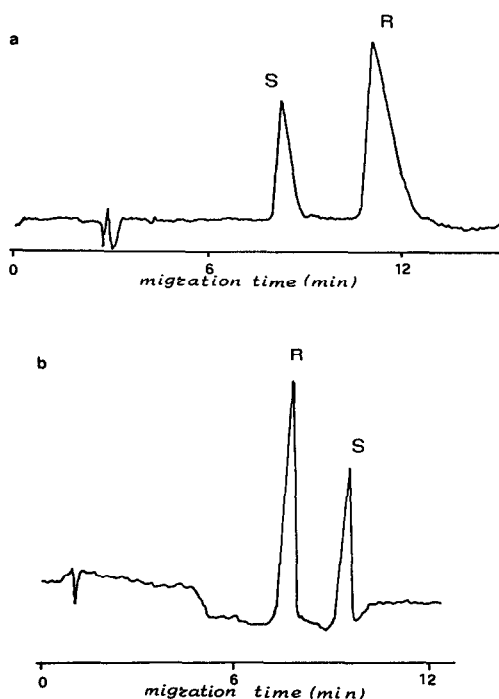
these results by different approaches such as washing the capillary.

## 4. Conclusions

We have demonstrated that a chargeable β-CD polymer can be advantageously employed for the enantiomeric resolution of basic compounds of pharmaceutical interest in a relatively short time (less than 5 min). Resolution and complexation are influenced by the amount of chiral polymer (generally increasing with increasing amount of chiral additive), the shape of the analyte molecule and the pH of the background electrolyte. The chiral additive can be easily used in a wide pH range, giving the opportunity to control both the electroosmotic flow and the charge of the cyclodextrin polymer. The latter effect is very important in order to enhance the ion-pairing effect with the analytes and reverse the migration order, which is important when the minor component in a enantiomeric mixture has to be determined.

In order to select the optimum electrophoretic separation conditions, the composition of the BGE has to be selected so as to avoid the use of a pH and a poly-β-CD concentration such that the mobility of the enantiomers is close to that of the electroosmotic flow.

Owing to the use of a limited amount of electrolyte (vials of 500 $\mu$l are used with the electrophoresis apparatus), this method is cheap in comparison with others where expensive stationary phases are required.

Further studies will be carried out in order to verify the effect of organic modifiers on the resolution of enantiomeric compounds.



Fig. 9. Electrophoretic separation of a mixture containing (S)- and (R)-propranolol (1:2). Capillary: 17 cm × 0.025 mm I.D. (coated); background electrolyte 0.05 $M$ acetate buffer (pH 4.5) and (a) 10 and (b) 20 mg ml$^{-1}$ of poly-β-CD; applied voltage, 12 kV; current, 20 $\mu$A; injection, pressure 10 psi corresponding to $35 \cdot 10^{-3}$ MPa for 2 s. Analytes were moving towards (a) the cathode (−) and (b) the anode (+).

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