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# Resolution improvement by use of carboxymethyl-β-cyclodextrin as chiral additive for the enantiomeric separation of basic drugs by capillary electrophoresis[1]

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**Abstract**

Three β-cyclodextrin derivatives—carboxymethyl-, dimethyl- and hydroxypropyl-β-cyclodextrin—were tested as chiral selectors for the enantioseparation of seven basic drugs in free solution capillary electrophoresis, using buffers made of 100 mM phosphoric acid adjusted to pH 3.0 with triethanolamine in fused silica capillaries thermostatted at 15°C. The best results with respect to chiral resolution were obtained with carboxymethyl-β-cyclodextrin (CMCD): the enantiomers of all compounds examined were completely resolved with this β-cyclodextrin derivative. The influence of the CMCD concentration on the migration times, the apparent electrophoretic mobility difference and the resolution of the drug enantiomers was investigated thoroughly. Particularly impressive resolution values, up to 23.7, were obtained for several compounds in these capillary electrophoretic systems, using CMCD in the 5–15 mM concentration range.

*Keywords:* Capillary electrophoresis; Carboxymethyl-β-cyclodextrin; Chiral basic drugs; Derivatized cyclodextrins; Enantiomeric separations

## 1. Introduction

The separation of the enantiomers of physiologically active chiral compounds and their individual determinations is of prime importance in pharmacokinetic, clinical and toxicological studies. Chirally selective methods are also required for testing the enantiomeric purity of bulk pharmaceuticals and other xenobiotics such as herbicides, produced as single enantiomers, and for controlling the chiral stability of formulations.

Besides chromatographic techniques, capillary electrophoresis (CE) has proved to be a powerful tool for the separation of enantiomers, providing very high efficiency, short analysis times, low operational cost and fast method development. The number of papers dealing with applications of CE in chiral analysis has increased exponentially over

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the past few years and several reviews have recently been published in this field [1–7]. In CE, enantiomeric separations have been achieved predominantly in free solution, using cyclodextrins (CDs) and their derivatives as chiral selectors [8–31]. Models showing the effect of CD concentration on the difference in apparent electrophoretic mobility between the enantiomers [8] and resolution [9,10] have been described, as well as more complex models also involving the influence of the buffer pH on enantioselectivity [11,12] and resolution [13].

A number of chiral drugs and related compounds have been enantioseparated by addition of $\beta$-CD, $\gamma$-CD or uncharged $\beta$-CD derivatives to acidic buffers [8–16,18–27,29,31]. These conditions are particularly favourable for the resolution of basic compounds in uncoated fused silica capillaries, since these compounds are present in cationic form and the electroosmotic flow (EOF) is minimized. A suppression of the EOF and of analyte interactions with the capillary wall can be obtained by modifying the surface by dynamic coating with hydrophilic polymers, such as hydroxyalkylcelluloses [11–16] or poly(vinyl alcohol) [14,16], which usually gives rise to an improvement in peak symmetry and efficiency. Capillaries permanently coated with polyacrylamide [17–19] or poly(vinyl alcohol) [14] have also been used. Alkylammonium ions, such as triethanolamine [20–22], triethylamine [20,23,24], tetraalkylammonium ions [15,18,25,26] or polyamines [18], used as cationic components of the buffer, or a cationic polymer, such as Polybren [14], can also be adsorbed to the capillary wall, leading in most cases to a reversal of the EOF [20–23,25–26].

Ionizable $\beta$-CD derivatives, and in particular those containing carboxy groups, such as carboxymethyl-, carboxyethyl- or succinyl-$\beta$-CD [17–19,21,22,27] have also been used as chiral additives in CE. They often provide higher flexibility in the optimization of enantiomeric resolution, mainly due to the possibility of changing their charge and hence their electrophoretic mobility by altering the pH of the running buffer. By using such CD derivatives in the charged form, neutral compounds can also be enantio-separated [17]. Moreover, a reversal of the migration order of the enantiomers of charged solutes can often be achieved with these CD derivatives by switching from the uncharged to the charged form, which is of particular interest in enantiomeric purity testing [17,18]. Another charged CD derivative, the polyanionic sulfobutyl ether $\beta$-CD, was also found to be useful in the enantioseparation of cationic [15,16,19,22,24,27–30], neutral [19,28–30] and even anionic compounds [19,30].

In previous papers [20,21] the usefulness of 0.1 M phosphate buffers adjusted to pH 3 triethanolamine and containing $\beta$-CD or one of its derivatives for the CE enantioseparation of a number of basic drugs was demonstrated. Triethanolamine was found to be a particularly suitable buffer co-ion for cationic analytes, giving rise to peaks with good symmetry, high efficiency and reproducible migration times. Guidelines for a rapid optimization of enantiomeric separations of basic compounds using such buffers in fused silica capillaries thermostatted at 15°C were proposed [20]. First, a screening of the different CDs tested was preformed at the same CD concentration. Second, the optimum concentration was determined for the CD giving the highest resolution in the screening test. Third, for analytes with a very high affinity for the CD, a further improvement in resolution could possibly be obtained by addition of an organic modifier, such as methanol, to the buffer. Among the neutral CD derivatives tested, heptakis (2,6-di-*O*-methyl)-$\beta$-CD (DMCD) and hydroxypropyl-$\beta$-CD (HPCD) were found to give the highest resolution values in most cases [20]. For propranolol, CD derivatives containing carboxy groups were also tested under the same conditions and a particularly high resolution value of 4.4 was obtained with carboxymethyl-$\beta$-CD (CMCD), making it possible to test the enantiomeric purity of propranolol at the 0.1% level [21].

In this work, three of the most effective CD derivatives, DMCD, HPCD and CMCD, were tested as chiral selectors for the enantiomeric separation of seven basic drugs selected as model compounds, using pH 3.0 phosphate–triethanolamine buffers in fused silica capillaries thermostatted at 15°C. The effects of the concen-

tration of CMCD on the migration behaviour of the enantiomers, the difference in their apparent electrophoretic mobilities and their resolution were investigated in particular. Conditions giving maximum enantiomeric resolution were deduced for the different basic drugs examined.

## 2. Materials and methods

### 2.1. Apparatus

All experiments were performed on a Hewlett Packard $^{3D}$CE instrument (Hewlett Packard, Palo Alto, CA) equipped with a diode-array detector, an automatic injector, an autosampler and a temperature-control system (15–60°C ± 0.1°C). An HP Vectra 486/66XM was used for instrument control and data handling. Electropherograms were printed on a HP Laser Jet 4 printer. A column cartridge was obtained from Hewlett Packard. The pH of buffers was adjusted by means of a model Delta 345 pH meter from Mettler (Halstead, UK).

### 2.2. Chemicals and reagents

Phosphoric acid (85%) and triethanolamine were of p.a. quality from Merck (Darmstadt, Germany). Water was of Milli-Q quality (Millipore Corporation, Bedford, MA). DMCD and HPCD were obtained from Janssen Chimica (Geel, Belgium). CMCD was from Cyclolab (Budapest, Hungary). Terbutaline sulfate, chlorpheniramine maleate and bupivacaine hydrochloride racemates were obtained from Sigma Chemical Company (St. Louis, MO). Isoprenaline sulfate racemate was from Ludeco (Brussels, Belgium), Dimethindene maleate, ephedrine hydrochloride and fenfluramine hydrochloride racemates were gifts from diverse sources. All compounds were used without further purification.

### 2.3. Electrophoretic technique

Capillary electrophoretic separations were carried out with uncoated fused silica capillaries (48.5 cm × 50 μm i.d., 40 cm to the detector). Before use, the capillary was treated successively with basic solutions (i.e. 1 M NaOH followed by 0.1 M NaOH), water and running buffer. The latter consisted of 0.1 M phosphoric acid adjusted to pH 3.0 with triethanolamine (0.084 M). At the beginning of each working day, the capillary was washed with separation buffer for 10 min and after each sample injection a washing of the capillary with buffer for 3 min was performed.

The applied voltage was 25 kV and UV detection was performed at 210 nm. Injections were made using the hydrodynamic mode (injection pressure: 5 kPa) for 2 s. The capillary was thermostatted at 15°C. The standard solutions were prepared by dissolving salts of racemic drugs in water at a concentration of 50 μg ml$^{-1}$.

The resolution ($R_s$) and plate number ($N$) were calculated according to the standard expressions based on peak width at half-height [32].

## 3. Results and discussion

### 3.1. Buffer composition and pH

All enantiomeric separations reported were performed at 15°C using buffers made of 100 mM phosphoric acid adjusted to pH 3.0 with triethanolamine and containing one CD derivative [20–22]. Under these conditions, triethanolamine was adsorbed to the capillary wall and gave rise to a reversal of the EOF, as observed with other alkylammonium ions added to the running buffer,

Table 1
Influence of cyclodextrin type on chiral resolution ($R_s$) (Buffer: 5 mM CD in 100 mM phosphoric acid adjusted to pH 3.0 with triethanolamine. Other conditions as described in Section 2)

| Analyte | DMCD | HPCD | CMCD |
|---|---|---|---|
| Bupivacaine | 2.3 | –[a] | 1.3 |
| Chlorpheniramine | <0.7 | 2.1 | 16.8 |
| Dimethindene | <0.7 | 2.1 | 13.3 |
| Ephedrine | 1.8 | 0.9 | 3.9 |
| Fenfluramine | 1.4 | –[a] | 1.4 |
| Isoprenaline | 4.4 | 1.8 | 3.6 |
| Terbutaline | 7.2 | 6.2 | 10.4 |

[a] No chiral resolution observed ($R_s$ <0.5).

such as short-chain tetraalkylammonium ions [25,26] or polyamines, like spermine [18]. At pH 3.0, the electroosmotic mobility, $\mu_{eo}$, had a low and fairly constant value of $-4.0 \times 10^{-5}$ $cm^2$ $V^{-1}$ $s^{-1}$ (EOF toward the anode), leading to highly reproducible migration times [21]. This electroosmotic mobility did not seem to be significantly influenced by the addition of a CD derivative to the buffer since the same value was obtained in the presence and in the absence of CD. A different behaviour was observed with triethylamine [23,24] and long-chain quaternary ammonium ions like cetyltrimethylammonium ion [18,25] which led to a reversal of the EOF only in the absence of CD. This can be explained by the strong tendency of these more hydrophobic cations to be included in CDs [18,24,25]. It should be noted, however, that the reversal of the EOF observed with triethanolamine was strongly pH-dependent since it was only obtained at a pH of around 3 (M. Fillet and J. Crommen, in preparation).

The use of pH 3.0 buffers with triethanolamine as cationic component leads to a resolution enhancement for the enantiomers of basic drugs, as can be seen from the resolution expression [2,4,9,10,13,15,25,33]:

$$R_s = 0.177 \cdot \Delta\mu_{ep} \cdot \left[ \frac{V}{D(\mu_{eo} + \bar{\mu}_{ep})} \cdot \frac{l}{L} \right]^{1/2} \quad (1)$$

where $\Delta\mu_{ep}$ and $\mu_{ep}$ are the difference and average of the apparent electrophoretic mobilities of the two enantiomers respectively, $D$ is the average diffusion coefficient, $V$ is the applied voltage and $L$ and $l$ are the total and effective (from the injection end to the detector) lengths respectively. Clearly, the resolution is increased when the analyte enantiomers still migrate towards the cathode, even though the EOF is directed towards the anode (negative $\mu_{eo}$), as is the case here. However, this resolution improvement will be obtained at the expense of migration times.

The best results with respect to peak symmetry and peak efficiency were also obtained around pH 3 with phosphate–triethanolamine buffers ([20]; M. Fillet and J. Crommen, in preparation), probably because the possibilities of interactions between the cationic analytes and the capillary wall were minimized under these conditions. Therefore changes of pH in the range 2–6 seem to be of no interest in such CE systems when uncharged CDs or CD derivatives are used as chiral selectors for the enantioseparation of basic compounds. With ionizable CD derivatives, such as those containing carboxy groups, an increase in pH can have a favourable effect on the enantiomeric resolution of cationic analytes but only if the EOF, directed towards the cathode at higher pH, and analyte–wall interactions are efficiently suppressed by modifying the capillary surface by either dynamic or permanent coatings ([14,17,18]; M. Fillet and J. Crommen, in preparation).

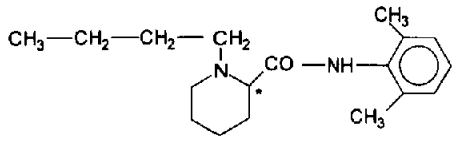
### 3.2. Influence of the CD type

CMCD was tested as chiral selector for the enantiomeric resolution of seven basic drugs with widely differing structural features (Fig. 1) and pharmacological properties, injected as racemates, and chiral resolution values were compared with those obtained at the same concentration with DMCD and HPCD which were found to be particularly useful for the CE enantioseparation of cationic analytes [20].

As can be seen from Table 1, large differences in chiral resolution were obtained with the three different CD derivatives at 5 mM concentration. HPCD seems to be the least effective chiral selector under these conditions since the corresponding resolution values were lower in most cases, no resolution ($R_s < 0.5$) was observed for two compounds (bupivacaine and fenfluramine) and only a partial resolution for ephedrine enantiomers. With the two other CD derivatives, chiral resolution was obtained for all compounds but in the case of DMCD the enantiomers of two compounds (chlorpheniramine and dimethindene) were only partially resolved so that no precise determination of resolution could be made ($R_s < 0.7$).

The best results with respect to chiral resolution were obtained with CMCD: an almost enantiomeric resolution was observed for all compounds ($R_s \geq 1.3$) and particularly high resolution values ($R_s > 10$) were obtained for chlorpheniramine, dimethindene and terbutaline enantiomers,

(1)



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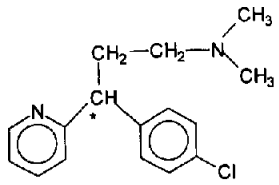

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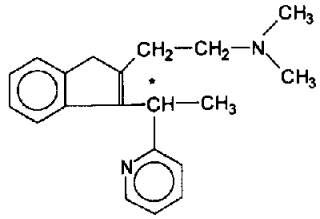

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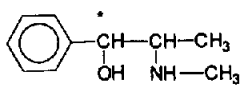

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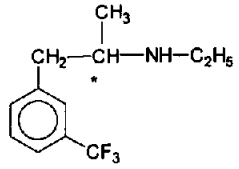

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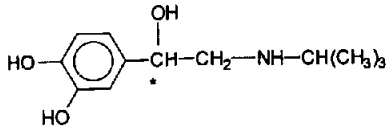

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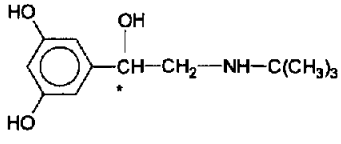
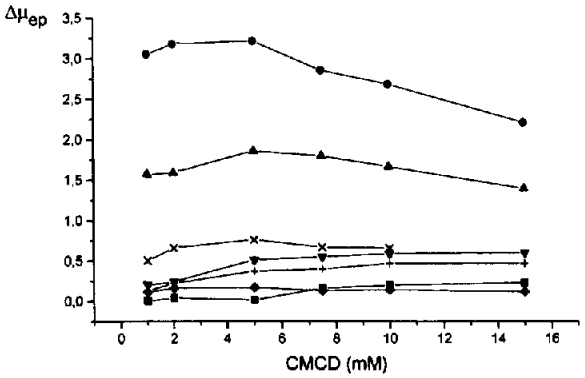
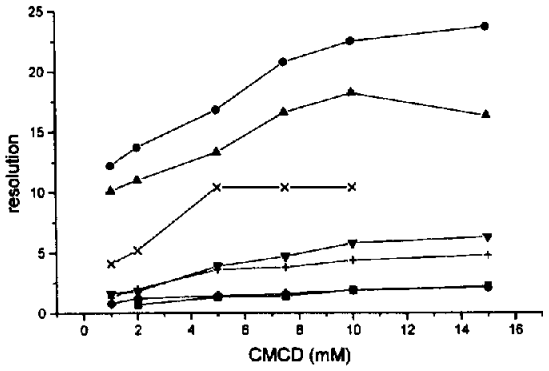


Fig. 1. Structures of (1) bupivacaine; (2) chlorpheniramine; (3) dimethindene; (4) ephedrine; (5) fenfluramine; (6) isoprenaline; (7) terbutaline.

in spite of the rather low CD concentration used in these preliminary experiments. These results confirm the high effectiveness of CMCD for the enantioseparation of basic compounds, in accordance with earlier observations made with propranolol [21] and other $\beta$-blockers (M. Fillet and J. Crommen, in preparation). It is likely that this very high enantioselectivity is at least partly related to a slight deprotonation of the carboxy groups of CMCD at pH 3.0, giving rise to stronger interactions with cationic analytes and to an electrophoretic migration of CMCD towards the anode, which should increase the difference in




(a)




(b)

Fig. 2. (a) Influence of carboxymethyl $\beta$-cyclodextrin concentration on apparent electrophoretic mobility difference ($\Delta\mu_{ep}$). (b) Influence of carboxymethyl $\beta$-cyclodextrin concentration on resolution ($R_s$). Buffer: 1–15 mM CMCD in 100 mM phosphoric acid adjusted to pH 3.0 with triethanolamaine. Other conditions as described in Section 2. Solutes: (■) bupivacaine; (●) chlorpheniramine; (▲) dimethindene; (▼) ephedrine; (◆) fenfluramine; (+) isoprenaline; (×) terbutaline.

Table 2
Influence of CMCD concentration on migration times (Buffer: 1–15 mM CMCD in 100 mM phosphoric acid adjusted to pH 3.0 with triethanolamine. Other conditions as described in Section 2)

| Analyte | CMCD concentration (mM) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 5 | 7.5 | 10 | 15 |
| Bupivacaine | 10.30 | 10.43/10.46 | 11.46/11.61 | 11.89/12.06 | 11.89/12.11 | 12.68/12.97 |
| Chlorpheniramine | 7.82/9.59 | 8.55/10.82 | 13.01/19.25 | 14.88/22.19 | 17.53/27.57 | 19.38/29.08 |
| Dimethindene | 6.91/7.54 | 7.67/8.47 | 11.94/14.42 | 15.80/20.23 | 15.95/20.08 | 19.33/24.43 |
| Ephedrine | 7.97/8.07 | 8.54/8.68 | 10.70/11.17 | 12.71/13.43 | 13.60/14.50 | 15.89/17.16 |
| Fenfluramine | 10.85/10.95 | 14.39/14.65 | 22.45/23.13 | 28.09/28.94 | 31.73/32.79 | 41.76/43.38 |
| Isoprenaline | 10.14/10.25 | 11.45/11.67 | 13.29/13.82 | 14.51/15.19 | 15.08/15.94 | 16.62/17.69 |
| Terbutaline | 12.80/13.47 | 16.24/17.71 | 25.76/30.39 | 27.07/31.43 | 29.44/34.70 | –[a] |

[a] Not determined (migration times higher than 60 min).

electroporetic mobility between the free and complexed enantiomers [3].

It is interesting to note that terbutaline gives high resolution values ($R_s > 6$) with the three CD derivatives. This is also true, to a lesser extent, for isoprenaline. These two compounds present similar structural characteristics which might be favourable for high chiral discrimination with these CD derivatives, i.e. the presence of two hydroxy groups on the benzene ring and an additional hydroxy group in the vicinity of the chiral center.

### 3.3. Influence of CD concentration

The dependence of the difference in apparent electrophoretic mobility between the enantiomers of the basic drugs, $\Delta\mu_{ep}$, on the concentration of CMCD, which gave by far the highest resolution values in preliminary experiments (cf. Table 1), was studied. Fig. 2(a) shows that, in accordance with the model developed by Wren and Rowe [8], the apparent mobility difference between the enantiomers of each compound reaches a maximum value at a given CMCD concentration. Four basic drugs (chlorpheniramine, dimethindene, fenfluramine and terbutaline) have maximum $\Delta\mu_{ep}$ values at CMCD concentrations around 5 mM while for the other three, maximum mobility differences are reached at much higher concentrations (15 mM or above), which should indicate that the enantiomers of the former drugs have a higher affinity for CMCD than those of the latter [8]. This seems to be confirmed by the fact that a much stronger increase in migration times was obtained for chlorpheniramine, dimethindene, fenfluramine and terbutaline enantiomers, as can be seen from Table 2. The CMCD concentration range was limited to 15 mM, because these compounds had too long migration times at higher concentrations. However, Fig. 2(b) shows that for these four compounds a maximum resolution is generally reached at higher CMCD concentrations than 5 mM. This can be explained by the fact that the decrease of the net mobility of these compounds, $\mu_{ep} + \mu_{eo}$, is more pronounced with increasing CMCD concentration, due to the increasing influence of the negative electroosmotic mobility (reversed EOF), and it can overcompensate for the decrease in $\Delta\mu_{ep}$ (cf. Eq. (1)). Under

Table 3
Conditions for maximum chiral resolution with CMCD (Buffer: 100 mM phosphoric acid adjusted to pH 3.0 with triethanolamine, containing CMCD. Other conditions as described in Section 2)

| Analyte | CMCD conc.(mM) | $\alpha$ | Resolution |
|---|---|---|---|
| Bupivacaine | 15 | 1.017 | 2.2 |
| Chlorpheniramine | 15 | 1.264 | 23.7 |
| Dimethindene | 10 | 1.160 | 18.2 |
| Ephedrine | 15 | 1.046 | 6.3 |
| Fenfluramine | 15 | 1.017 | 2.1 |
| Isoprenaline | 15 | 1.038 | 4.8 |
| Terbutaline | 5 | 1.093 | 10.4 |

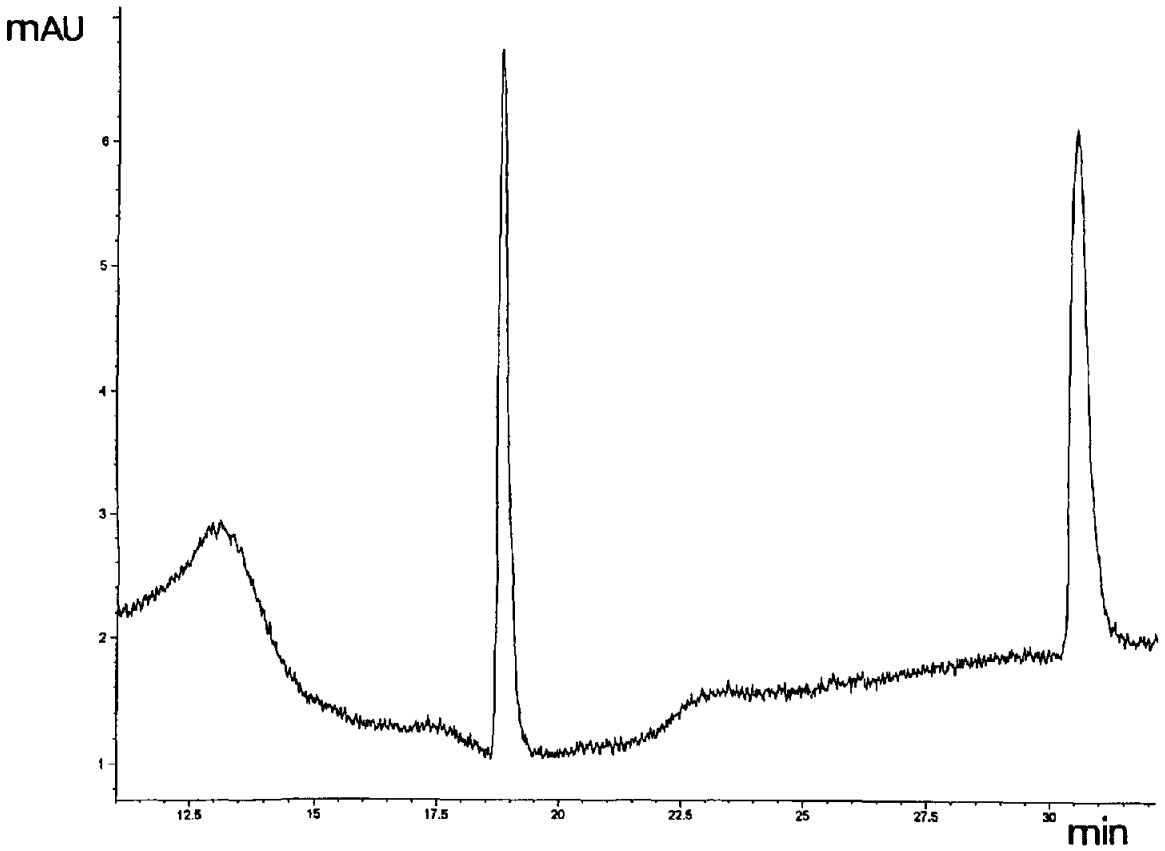



Fig. 3. Enantiomeric separation of chlorpheniramine. Buffer: 15 mM CMCD in 100 mM phorphoric acid adjusted to pH 3 with triethanolamine. Other conditions as described in Section 2.

these conditions, a further increase in resolution can be obtained at higher CMCD concentrations, at the expense of migration times. This is particularly the case for fenfluramine, which has about the same chiral resolution as bupivacaine at a 15 mM CMCD concentration although its $\Delta\mu_{ep}$ value is significantly lower, and for terbutaline, which has about the same $\Delta\mu_{ep}$ value as ephedrine at 10 mM but a much higher resolution value.

### 3.4. Conditions for maximum resolution

The highest resolution values which could be obtained with CMCD for the seven basic drugs examined, as well as the corresponding enantioselectivities, $\alpha$ (ratios of the apparent electrophoretic mobilities of the enantiomers), are listed in Table 3. As already seen in Fig. 2(b), these maximum $R_s$ values were found in most cases at 15 mM, i.e. the highest CMCD concentration in the range studied.

Resolution values higher than two were obtained for all compounds. Particularly impressive $R_s$ values of 23.7 and 18.2, among the highest ever reported in chiral CE, were observed with chlorpheniramine and dimethindene. This again confirms that the use of CMCD in combination with pH 3.0 phosphate–triethanolamine buffers at 15°C provides very effective CE conditions for the enantioseparation of basic compounds. It should be noted that only two compounds gave higher resolution values with another CD derivative (DMCD) under the same conditions: bupivacaine ($R_s$: 6.0; 30 mM DMCD) and isoprenaline ($R_s$: 5.6; 15 mM DMCD).

In general, there is a good correlation between enantioselectivity and resolution, the two highest $\alpha$ values being also given by chlorpheniramine and dimethindene. The high enantioselectivity obtained with these two compounds might be related to the presence in the vicinity of the chiral center of a pyridine ring which might interact selectively with the carboxy groups of CMCD. An even higher resolution could have been expected for chlorpheniramine, owing to the particularly high $\alpha$ value given by its enantiomers. However, as can be seen from Fig. 3, a relatively low peak efficiency ($N$: 60 000) was obtained for the enantiomers of this compound, in comparison to other analytes migrating in the same mobility range (typical $N$ values: 110 000–210 000), resulting in a negative influence on resolution.

Very high resolution values might be useful for the determination of drug enantiomers in complex matrices, such as biofluids, or for avoiding possibile interferences from other closely related substances in enantiomeric purity testing. However, this will generally require rather long analysis times (cf. Table 2). For other applications, $R_s$ values around 1.5 will generally be sufficient. Such values can already be obtained at low CMCD concentrations (1–2 mM) for most compounds, except bupivacaine (cf. Fig. 2(b)). Under these conditions, analysis times will be in the range 8–15 min range (cf. Table 2).

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