

## Enantioseparation in capillary electrophoresis using 2-hydroxypropyltrimethylammonium salt of $\beta$ -cyclodextrin as a chiral selector

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### Abstract

The resolving ability of the 2-hydroxypropyltrimethylammonium salt of  $\beta$ -cyclodextrin (TMA- $\beta$ -CD) as a chiral selector in capillary electrophoresis (CE) is reported in this work in continuation of our research on this subject [Chem. Soc. Rev., 25 (1996) 141; J. Chromatogr. A, 732 (1996) 143, 183]. The composition of the chiral selector and the effect of its addition to the background electrolyte on the EOF in a fused-silica and a polyacrylamide-coated capillary were studied. The enantioseparations of neutral chiral analytes are reported first using a positively charged cyclodextrin (CD) derivative. The carrier ability of a positively charged CD derivative is used for the migration of neutral analytes in a polyacrylamide-coated capillary. Several general advantages of this mode of electrophoretic separations are illustrated together with selected possibilities for the reversal of the enantiomer migration order for neutral and anionic analytes.

*Keywords:* Enantiomer Separation; Chiral selectors; Cyclodextrins; 2-Hydroxypropyltrimethylammonium- $\beta$ -cyclodextrin

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### 1. Introduction

Enantioseparation of neutral chiral analytes using charged cyclodextrin (CD) derivatives is one of the challenging fields of chiral capillary electrophoresis (CE) [1]. Anionic CD derivatives such as carboxymethyl and carboxyethyl ethers of  $\beta$ -CD [1–5], sulfobutyl ether of  $\beta$ -CD (SBE- $\beta$ -CD) [1,5,6], sulfobutyl ether of  $\beta$ -CD [1,5] and  $\beta$ -CD sulfate [7] are used exceptionally as chiral selectors in these separations. In contrast, no studies are described yet on

the use of cationic CD derivatives for enantioseparation of neutral chiral compounds. Moreover, the potential of positively charged CD derivatives remains scarcely studied not only in the separation of the enantiomers of neutral chiral analytes but also in chiral CE in general [1,8–13].

Some potential advantages of positively charged CD derivatives as chiral selectors in CE include the following: (a) enhanced attractive electrostatic interaction with anionic analytes; (b) relative simplicity of a design of a substitution degree and subsequently the self-mobility of chiral selector; (c) availability of synthetic techniques for the regiospecific and site-specific introduction of the positively charged groups onto the CD rim; (d) further modification of the

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chiral recognition ability by complexation of some cationic CDs with transition metals.

The use of positively charged TMA- $\beta$ -CD as a chiral selector for the enantioseparation of neutral and anionic chiral compounds is shown in this paper. The advantage of quaternary ammonium salts in comparison to other positively charged chiral selectors is that they possess a pH-independent self-electrophoretic mobility almost in the entire pH range acceptable in CE.

Neutral coating of the inner wall of a fused-silica capillary avoids the substantial interactions of the positively charged chiral selector with the capillary wall in this case but at the same time eliminates or drastically decreases the EOF. This necessitates that the chiral selector in addition to the enantioseparation also transports the resolved enantiomers to the detector. The use of charged CDs as carriers for neutral analytes in CE was first noted by Terabe [8].

The present study shows that TMA- $\beta$ -CD with an average substitution degree of 3.5 meets both of the above mentioned requirements, i.e., it can bind the neutral analytes stereoselectively and at the same time transport them through the capillary. It is worth mentioning that a CE-mode in which the transportation ability of the chiral selector is employed for the migration of the analytes to the detector may offer some advantages and complementary properties to that of the normal-mode CE. The first advantage is that no extra mobility exists in the former case and the analyte migrates in the direction of the detector only in the bonded form with a chiral selector, i.e., when the chiral recognition occurs. The second advantage is that an increase of the concentration of a chiral selector within a certain limit can lead simultaneously to both increased separation factor and increased peak efficiency. This dependence is complementary to that observed in normal-mode CE, where the increase of the concentration of a chiral selector can increase the separation factor but usually decreases the peak efficiency. The analysis times commonly increase with increasing the chiral selector concentration in normal-mode CE, whereas it decreases in a certain concentration limit (unless the increased viscosity of the background electrolyte controls the migration) with increasing the chiral selector concentration in the CE-mode with a chiral selector as the carrier.

All these effects together with several possibilities for the reversal of enantiomer migration order of neutral and anionic analytes are described in this work.

## 2. Experimental

### 2.1. Chemicals and reagents

Racemic ( $\pm$ )-hexobarbital and ( $\pm$ )-*N*-methylphenobarbital (mephobarbital) were purchased from Sigma (Deisenhofen, Germany). Imidazole, racemic ( $\pm$ )-benzoin, ( $\pm$ )-5-methyl-5-phenyl hydantoin and optically pure enantiomers of *R*-(-) and *S*-(+)-1,1'-binaphthyl-2,2'-diyl hydrogen phosphate were obtained from Aldrich (Steinheim, Germany). Racemic ( $\pm$ )-thalidomide was a gift from Pharma Grünenthal (Grünenthal, Germany). Thalidomide enantiomers were separated on a preparative scale by low-pressure liquid chromatography on a poly[(*S*)-*N*-(1-cyclohexylethyl)methacrylamide] stationary phase [14]. Methacrylic acid-3-trimethoxysilylpropylester, Tris(hydroxymethyl)aminomethane, boric acid, ethylenediaminetetraacetic acid (EDTA), acrylamide, sodium persulfate and *N,N,N',N'*-tetra-methylethylenediamine, all used for the preparation of polyacrylamide-coated capillaries [15], were purchased from Fluka (Buchs, Switzerland). 2-Hydroxypropyltrimethylammonium salt of  $\beta$ -CD (TMA- $\beta$ -CD) with an average substitution degree of 3.5 and carboxymethyl ether of  $\beta$ -CD (CM- $\beta$ -CD) with an average substitution degree of 3.5 were a gift from Wacker Chemie (Munich, Germany). Analytical grade  $\text{KH}_2\text{PO}_4$ ,  $\text{H}_3\text{PO}_4$ ,  $\text{H}_2\text{SO}_4$  and NaOH were obtained from Merck (Darmstadt, Germany).

### 2.2. Instrumentation

#### 2.2.1. Capillary electrophoresis

A Grom capillary electrophoresis system 100 (Herrenberg, Germany), equipped with Linear Instruments (Reno NV, USA) UV-Vis 200 detector and a HP 3396 A integrator (Hewlett-Packard, Avondale, PA, USA) was used with a fused-silica or a polyacrylamide-coated capillary (Grom) of 60 cm (43 cm effective length)  $\times$  50  $\mu\text{m}$  I.D.. The sample

was introduced hydrostatically (10 cm) during 5 s. Detection was carried out at 210 nm.

Electrophoretic analysis of TMA- $\beta$ -CD was performed in a polyacrylamide-coated capillary in imidazole buffer at pH 4.0. Peaks of TMA- $\beta$ -CD with different substitution degrees were detected indirectly at 205 nm.

### 2.3. Buffer and sample preparation

A stock solution of 50 mM  $\text{KH}_2\text{PO}_4$  was prepared in double distilled, deionized water. The pH was adjusted with 0.16 M  $\text{H}_3\text{PO}_4$  or 0.5 M NaOH. The run buffers were prepared accordingly after the addition of appropriate amounts of the chiral selectors. All solutions were filtered and degassed by sonication before use. Stock solutions of 1 mg/ml of the racemic solutes were prepared, stored at 4°C and diluted to 100–200  $\mu\text{g}/\text{ml}$  before use.

## 3. Results and discussion

### 3.1. Composition of the chiral selector

Most of the commercially available CD derivatives are mixtures of components with various degrees of substitution. The degree of substitution and the position of the substituents both may affect the chiral recognition ability of CDs as documented by several recent studies [16,17].

CE can provide some preliminary information about the composition of charged CD derivatives [1,5,18–21]. The electropherogram of TMA- $\beta$ -CD (Fig. 1) shows that this is a mixture of at least four charged components with different degrees of substitution. The fast atom bombardement (FAB) and matrix-assisted laser desorption–ionisation time of flight (MALDI–TOF) MS spectra confirmed the multicomponent composition of TMA- $\beta$ -CD, but showed some ghost peaks which complicated the peak assignment.

### 3.2. Effect of the TMA- $\beta$ -CD concentration on the electroosmotic flow (EOF)

Since TMA- $\beta$ -CD is a mixture of positively charged compounds a strong interaction of this chiral

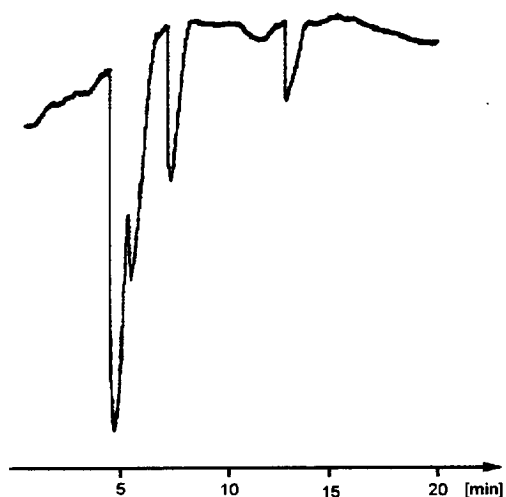
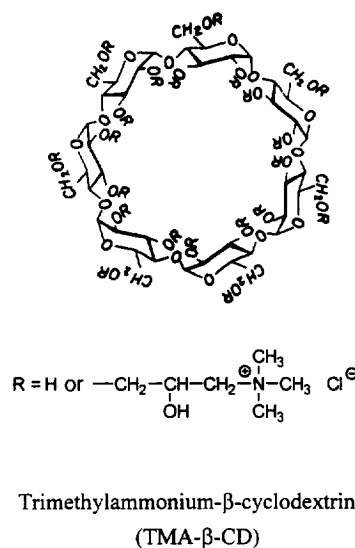


Fig. 1. Structure and electrophoretic profile of TMA- $\beta$ -CD. Conditions: 5 mM imidazole buffer pH 4.0, +500 V/cm, indirect detection at 205 nm, acrylamide-coated capillary (60/43 cm).

selector is possible with the oppositely charged fused-silica capillary wall. This was confirmed in the experiment performed at pH 6.0 using mesityl oxide as the EOF marker (Fig. 2). The addition even of a few  $\mu\text{g}$  of TMA- $\beta$ -CD to the background electrolyte markedly suppressed the EOF and no measurable EOF was observed in the buffer containing 1 mg/ml of TMA- $\beta$ -CD. The addition of this chiral selector in

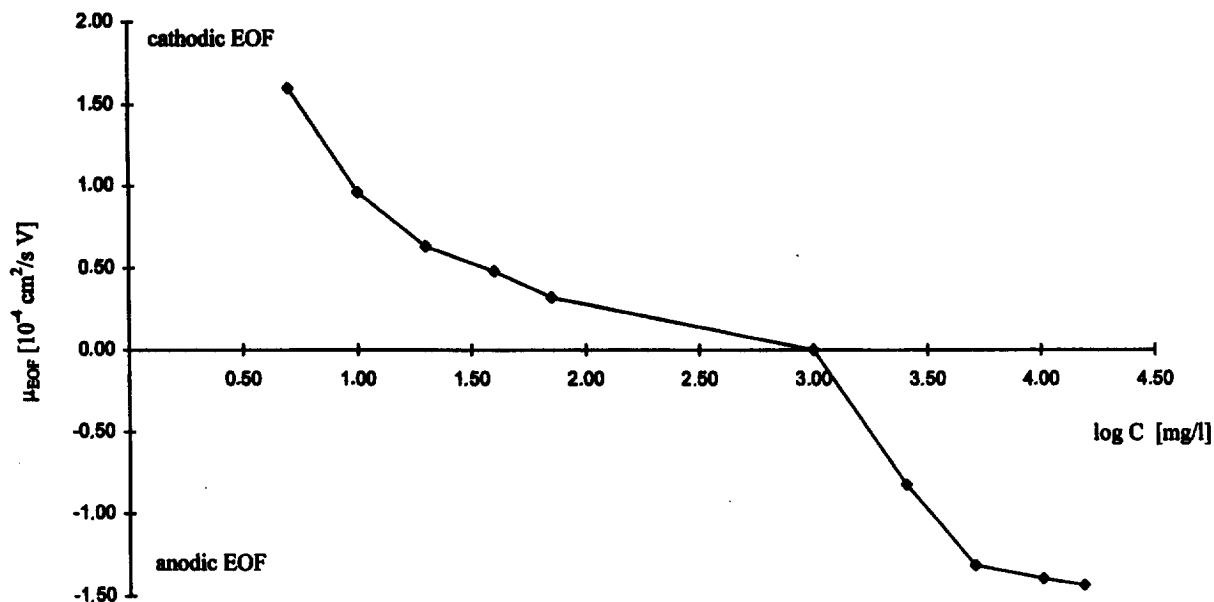


Fig. 2. Dependence of the EOF (mesityl oxide as the marker) on the concentration of TMA- $\beta$ -CD (mg/l) in the background electrolyte in a fused-silica capillary. Capillary, 60/43 cm; 50 mM phosphate buffer pH 6.0; applied voltage, +400 V/cm. Detection on the cathodic side of the capillary in the concentration range of TMA- $\beta$ -CD 0.005–0.1 mg/ml and on the anodic side of the capillary at a TMA- $\beta$ -CD concentration of 15 mg/ml.

the amount of 2.5 mg/ml to the background electrolyte resulted in a significantly reversed (anodic) EOF. As it is shown from these data TMA- $\beta$ -CD interacts strongly with the negatively charged fused-silica capillary wall and may cause the reversal of the EOF similar to positively charged surfactants.

It may also be possible that there are other kinds of solute interactions with the capillary wall apart from those of electrostatic nature. Therefore, the effect of the addition of TMA- $\beta$ -CD to the background electrolyte on the generation of the anodic EOF was also studied in the polyacrylamide coated capillary. Neither measurable anodic nor cathodic EOF was detected in this capillary at pH 6.0 unless TMA- $\beta$ -CD was added to the background electrolyte. After the addition of TMA- $\beta$ -CD an extremely low anodic EOF was observed only at the concentration of TMA- $\beta$ -CD as high as 20 mg/ml (the migration times of mesityl oxide were 72 and 69 min at pH 3.0 and 6.0, respectively). These data indicate that no significant adsorption of TMA- $\beta$ -CD occurs on the neutral capillary wall.

### 3.3. Enantioseparation of neutral chiral analytes in CE using TMA- $\beta$ -CD

Enantioseparation of neutral chiral analytes in CE is mainly performed using anionic CD derivatives [1–7], chiral micelles [8,22,23] or using a combination of CDs and chiral or achiral micelles [23]. These separations are performed in uncoated silica capillaries and the electroosmotic flow (EOF) is used as a driving force for neutral analytes which possess no electrophoretic mobility. As mentioned earlier the potential of charged CD derivatives as carriers for neutral analytes was first noted by Terabe [8]. Schmitt and Engelhardt had used negatively charged carboxymethyl ether of  $\beta$ -CD (CM- $\beta$ -CD) for the observation of the pH dependent reversal of enantiomer migration order of ( $\pm$ )-ephedrine as well as a driving force for neutral chiral analytes in a coated silica capillary [4].

Mazzeo et al. [22] used the carrier capability of a negatively charged chiral surfactant, (*S*)-2-(dodeoxycarbonylamino)-3-(*S*)-methyl-1-sulfoxyptane to

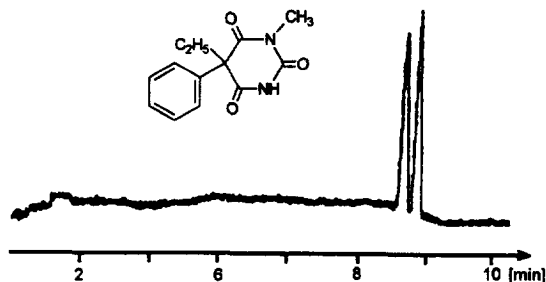


Fig. 3. Enantioseparation of ( $\pm$ )-*N*-methylphenobarbital using 5 mg/ml TMA- $\beta$ -CD as a chiral selector. Conditions: 50 mM phosphate buffer pH 3.0; +400 V/cm; detection 210 nm; acrylamide-coated capillary (60/43 cm).

observe the pH dependent reversal of the enantiomer migration order of the neutral chiral compound benzoin. Agyei et al. [24] employed the carrier ability of dextran sulfate to achieve the detection of the cationic chiral analyte chlorpheniramine on the anodic side of the separation capillary.

The carrier capability of the positively charged CD derivative TMA- $\beta$ -CD was used in this study for the first time as driving force for neutral chiral analytes. Figs. 3 and 4 show the enantioseparation of the chiral barbiturates *N*-methylphenobarbital and hexobarbital using TMA- $\beta$ -CD in the concentrations of 5 and 10 mg/ml, respectively. The migration times in these cases provide useful information for a

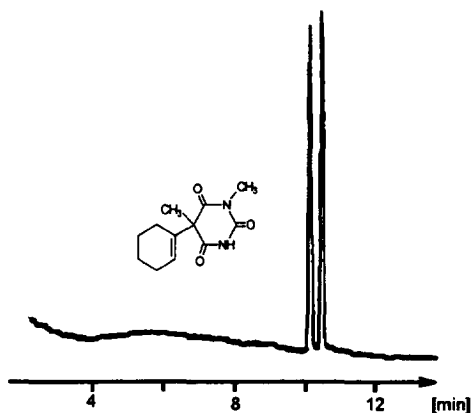


Fig. 4. Enantioseparation of ( $\pm$ )-hexobarbital using 10 mg/ml TMA- $\beta$ -CD as a chiral selector. Conditions as in Fig. 3.

calculation of binding constants of enantiomers with the chiral selector. The preferentially bonded enantiomer will migrate with higher velocity because no other driving force except that of transportation of the analyte with the chiral selector exists in this system. All these separations were performed in a polyacrylamide coated capillary. Under these conditions, only a very small anodic EOF is generated in this capillary after the addition of a relatively high amount of TMA- $\beta$ -CD as illustrated above.

In order to obtain nearly the same migration times for both *N*-methylphenobarbital and hexobarbital (Figs. 3 and 4) a 2-fold increase in the concentration of the chiral selector for hexobarbital was required. This indicates that *N*-methylphenobarbital is complexed more strongly with the TMA- $\beta$ -CD. The stronger interaction of the phenyl moiety of *N*-methylphenobarbital with the hydrophobic cavity of TMA- $\beta$ -CD compared to the cyclohexyl moiety of hexobarbital might be the reason for this difference observed in the CE experiment.

Fig. 5 shows the simultaneous enantioseparation of ( $\pm$ )-benzoin and ( $\pm$ )-5-methyl-5-phenylhydantoin using 25 mg/ml TMA- $\beta$ -CD. Baseline separations are achieved for both racemates. The shorter migration time of ( $\pm$ )-benzoin is again an evidence for the more tight interaction of this solute with

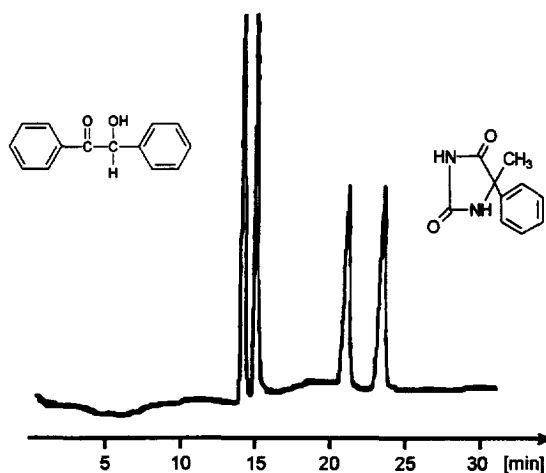


Fig. 5. Enantioseparation of ( $\pm$ )-benzoin and ( $\pm$ )-5-methyl-5-phenylhydantoin using 25 mg/ml TMA- $\beta$ -CD as a chiral selector. Conditions as in Fig. 3.

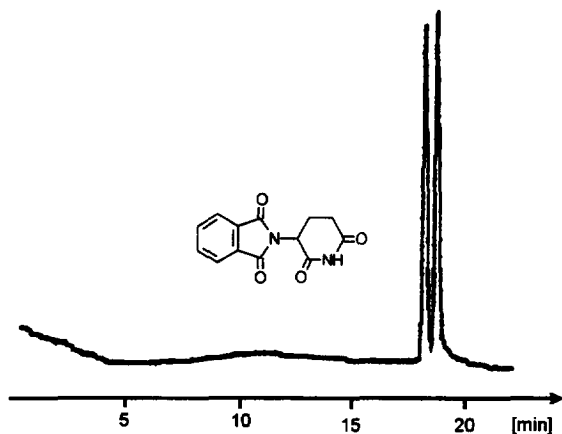


Fig. 6. Enantioseparation of (±)-thalidomide using 20 mg/ml TMA-β-CD as a chiral selector. Conditions as in Fig. 3.

TMA-β-CD than that of 5-methyl-5-phenyl hydantoin. As an additional example, the baseline separation of (±)-thalidomide with 20 mg/ml TMA-β-CD as a buffer additive is shown in Fig. 6.

### 3.4. Effect of TMA-β-CD concentration on the chiral resolution of the enantiomers of hexobarbital

The effect of the concentration of TMA-β-CD on the separation parameters (separation factor  $\alpha$ , peak efficiency  $N$ , resolution  $R_s$  and analysis time) of the hexobarbital enantiomers was studied. As shown in Fig. 7 the peak efficiency ( $N$ ) increased continuously with increasing TMA-β-CD concentration. The separation selectivity decreases with the increase in TMA-β-CD concentration. Peak resolution ( $R_s$ ) increased initially, reaching a maximum and then decreased. The analysis time decreased continuously with increasing TMA-β-CD concentration.

### 3.5. Reversal of the enantiomer migration order of neutral and anionic analytes using TMA-β-CD

The use of oppositely charged chiral selectors with the same chiral recognition pattern in the analysis of

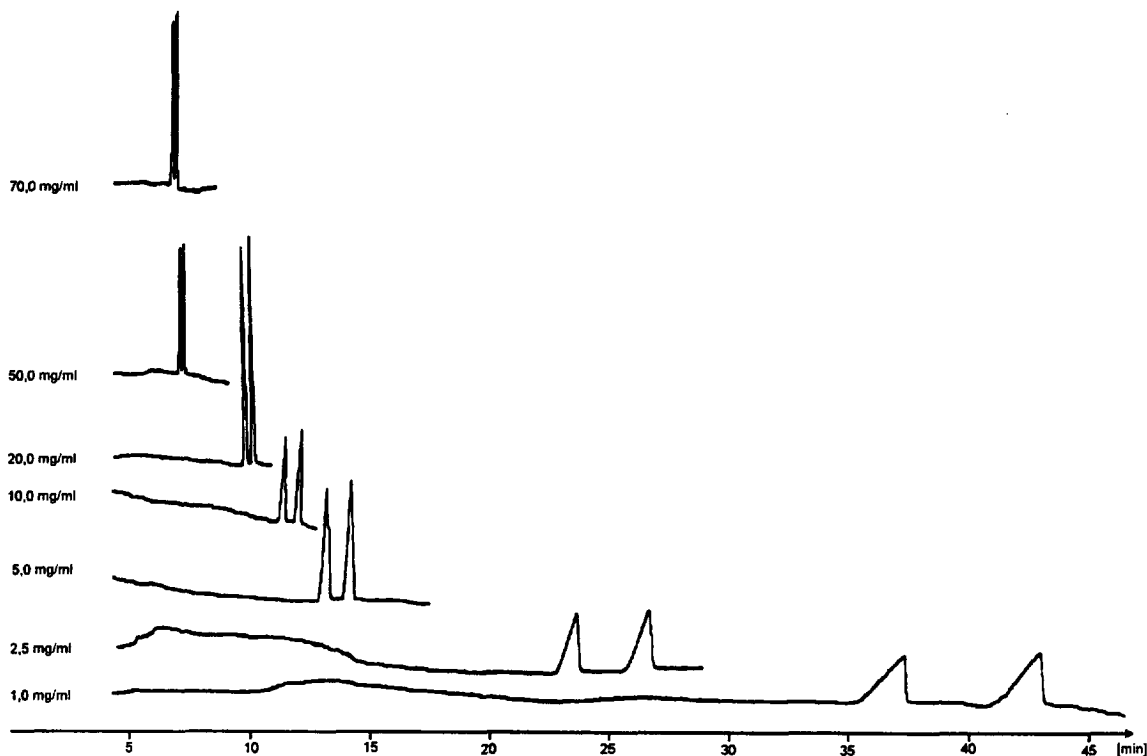


Fig. 7. Enantioseparation of (±)-hexobarbital using various concentrations of TMA-β-CD as a chiral selector. Conditions as in Fig. 3.

neutral analytes in a fused-silica capillary should result in the opposite migration order of enantiomers. This is well documented by Mayer et al. [25]. The migration order of the neutral chiral analyte thalidomide is  $R(+)$  before  $S(-)$  when resolved with the anionic CD derivative carboxymethyl ether of  $\beta$ -CD (CM- $\beta$ -CD) (Fig. 8, top). The addition of even very small amounts (0.005–1.0 mg/ml) of

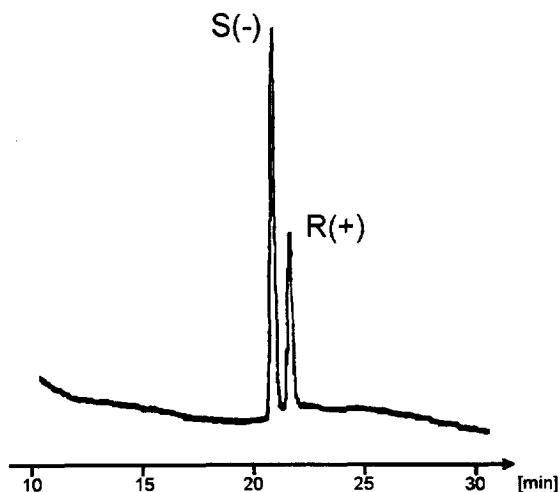
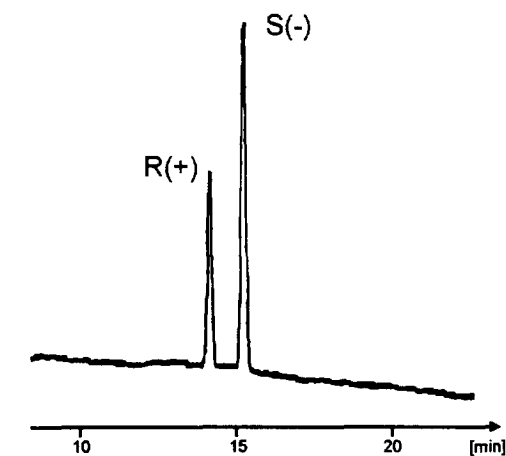


Fig. 8. Enantioseparation of ( $\pm$ )-thalidomide [ $R(+)$ : $S(-)$ ]=1:2] with 20 mg/ml CM- $\beta$ -CD (top) and 20 mg/ml TMA- $\beta$ -CD (bottom). Conditions (top): 50 mM phosphate buffer pH 6.0, +400 V/cm, detection at the cathodic end; fused-silica capillary (60/43 cm). Conditions (bottom): 50 mM phosphate buffer pH 3.0, +400 V/cm, detection at the cathodic end; acrylamide-coated capillary (60/43 cm).

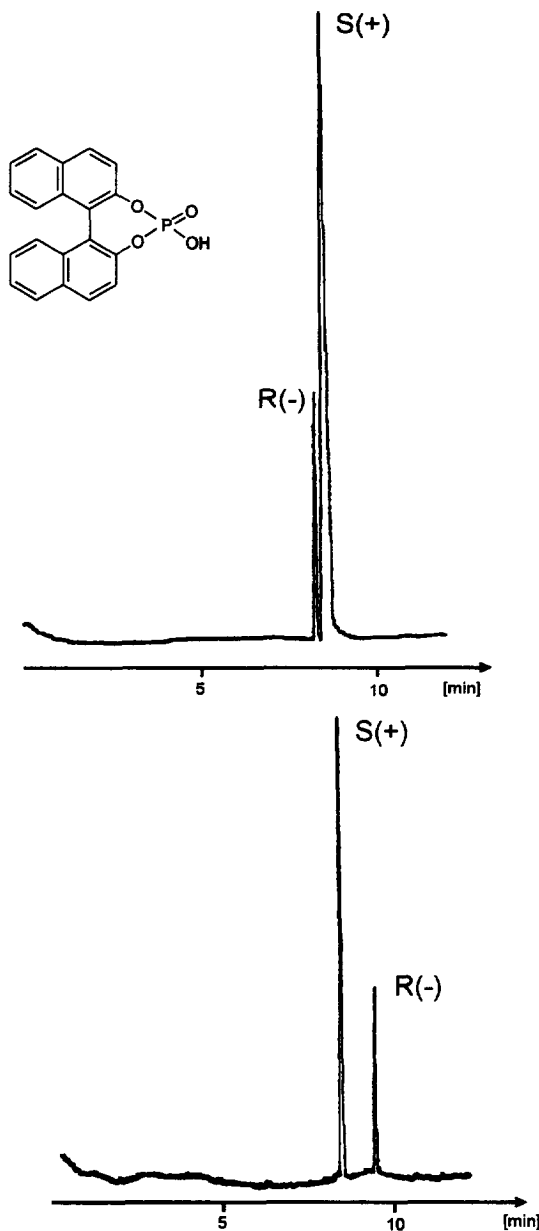


Fig. 9. Enantioseparation of ( $\pm$ )-1,1'-binaphthyl-2,2'-diyl-hydrogen phosphate [ $R(-)$ : $S(+)$ ]=1:3] with 0.05 mg/ml (top) and 10 mg/ml (bottom) TMA- $\beta$ -CD. 50 mM phosphate buffer pH 5.0, +400 V/cm, acrylamide-coated capillary (60/43 cm). Detection on the anodic side at a TMA- $\beta$ -CD concentration of 0.05 mg/ml and on the cathodic side at a TMA- $\beta$ -CD concentration of 10 mg/ml.

TMA- $\beta$ -CD in the background electrolyte at this pH drastically suppressed the EOF in a fused-silica capillary (Fig. 2) and made the detection of the neutral analyte impossible.

The reversal of the enantiomer migration order was observed in a polyacrylamide-coated capillary, when the amount of TMA- $\beta$ -CD is sufficient for both the separation and transport of thalidomide enantiomers to the detector in a reasonable time (Fig. 8, bottom).

Another example of the reversal of the enantiomer migration order of the anionic chiral compound 1,1'-binaphthyl-2,2'-diyl-hydrogen phosphate depending on the concentration of TMA- $\beta$ -CD is shown in Fig. 9. This effect is achieved with increasing the degree of complexation of the analyte due to increased TMA- $\beta$ -CD concentration in the background electrolyte. This allows to perform the detection of the analyte alternatively on the anodic (low concentration of TMA- $\beta$ -CD) or on the cathodic (high concentration of TMA- $\beta$ -CD) side of the separation capillary, by this way obtaining reversal of the migration order of enantiomers. Similar examples using the negatively charged CM- $\beta$ -CD polymer [26] or chiral micelle [22] have been already described in the literature.

Thus, positively charged TMA- $\beta$ -CD seems to be a very useful chiral selector for CE enantioseparations of anionic, neutral and even possible for positively charged analytes [1,10,11]. The mode of chiral CE, when the chiral selector in addition to chiral separation also facilitates the migration of analytes to the detector, offers some advantages and complementary properties to those of the normal mode CE.

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