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Optimization of enantiomeric separations in capillary electrophoresis by reversal of the migration order and using different derivatized cyclodextrins

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Abstract

In CE it is easy to optimize the elution order of enantiomeric pairs by reversal of the migration order. Several methods to achieve migration reversal for cationic as well as anionic enantiomers have been investigated. In principle three different approaches can be used. Firstly, electroosmotic flow can be reversed using different additives in the electrolyte buffer. Secondly, selecting different cyclodextrins as chiral additives can also reverse the migration order because of different separation and complexation mechanisms. Especially derivatized cyclodextrins have a great potential for this application and additionally contribute to the number of chiral selectors available in CE. In the third case, chargeable cyclodextrins offer the possibility for migration order reversal by varying the pH value.

1. Introduction

The separation of enantiomers has been a challenging area of research in the field of separation science. Nowadays many analytical methods are available for the separation of enantiomers such as HPLC, supercritical fluid chromatography (SFC), GC and recently CE. CE has already been established for chiral analysis. This is reflected by many reviews and applicative work in this field [1–5]. The reasons for the fast breakthrough are certainly low analysis costs, fast method development and easy handling of ionic or ionogenic analytes without previous derivatisation. In order to expand the applicability of the analysis of chiral compounds by CE the search for new types of chiral selectors is still a main area of research. Terabe et al. [6] introduced micelle forming detergents for the separation of enantiomers. Fanali and co-workers [3,7] employed derivatized neutral and cationic cyclodextrins as chiral selectors. Smith [8] and Schmitt and Engelhardt [9,10] used derivatized anionic cyclodextrins for the separation of neutral and cationic enantiomers. Bile salts were introduced by Nishi et al. [11]. Birnbaum and Nillson [12] and Valtcheva et al. [13] focused on proteins as chiral additives. Aiken and Huie [14] used small chiral compounds incorporated into a micelle to generate a chiral selector. Dextrins were introduced by D'Hulst and Verbecke [15] for the separation of enantiomers. Chiral stationary phases bound to the capillary wall were investigated by Meyer and Schurig [16].

The reversal of the migration order of enantiomers is also an important issue, especially when

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impurities have to be detected and the mobility differences to the main peak are small. As in HPLC, SFC and GC the main component can disturb the analysis of the minor component in the case of overloading of the system or because of tailing peaks. Therefore it is advantageous to detect impurities as the first migrating peak in a chromatogram. With chromatographic methods this can only be achieved by choosing another stationary phase i.e. preparing another column with the other enantiomer which might be tedious and expensive. Asymmetrical peaks in CE are a result of two different mechanisms. The first one is based on slow adsorption–desorption equilibria of the analyte and the capillary wall resulting always in a tailing peak. The second one is due to electrodispersion coming from conductivity-mismatch between the sample zone and the surrounding buffer. This results in triangular fronting or tailing analyte peaks because the mobility of the buffer electrolyte does not match to the mobility of the analyte. Also the concentration of the buffer can be too low and the observed peak shape is a result of overloading. Triangular peaks generated by a non-optimized background electrolyte (buffer) will only be briefly discussed. So it is very important in the determination of optical purity to change easily the elution order to have the trace component on the asymmetric side of the peak.

One elegant way for reversal of the migration order for any analyte is to reverse the electroosmotic flow (EOF). Many different ways have been described in literature so far. Huang et al. [17], Jones and Jandik [18] and Yao et al. [19] used long-chained cationic surfactants for the reversal of the EOF via formation of a double layer on the capillary surface. Adsorbed cationic polyamines were employed by Beck [20], and immobilized cationic coatings on the inner capillary wall were introduced by Towns and Regnier [21] and Huang et al. [22]. Also the application of a second external electric field outside the capillary has been described by Lee and coworkers [23,24]. The last two methods are somewhat critical. So far charged coatings on the capillary wall seem not to be stable over a long time. The use of a second external electric field needs a more difficult instrumental set-up and is still not available in any commercial instrument. The latter method works only at low pH values with low ionic strength of the running buffer.

Micellar electrokinetic chromatography (MEKC) is also a suitable tool for the reversal of the migration order as long as detergents of derivatized amino acids are used. These chiral selectors were introduced by Terabe and coworkers [25,26] and later improved by Mazzeo et al. [27]. Employing the pure (+) or the pure (−) detergent as micelle-forming agents, the migration order can easily be changed. As in HPLC, this method corresponds to the reversal of the chiral environment, and requires both types of enantiomeric detergents.

In this paper we will discuss the application possibilities of spermine, spermidine and cetyltrimethylammonium bromide (CTAB) as potential flow reversal agents together with cyclodextrins (CDs) as chiral selectors for the separation of anionic enantiomers. Furthermore, different derivatized CDs at different pH values were used for the separation of cationic enantiomers. In some cases the nature of the derivatisation as well as the buffer pH was found to revers the migration order of some enantiomers. Since many new types of CDs have been introduced here the nature of this paper is not only focused on the reversal of the migration order but also to expand the number of selectors in CE. The reversal of the migration order with the CD concentration has been already described elsewhere [10].

2. Experimental

Fused-silica capillaries (75 μm I.D.) were obtained from Polymicro Technologies (Phoenix, AZ, USA) and coated with 4% T [T = (g acrylamide + g N,N′-methylenebisacrylamide)/100 ml solution] linear polyacrylamide following a procedure described by Hjertén [28]. For coating the capillary was treated overnight with a 50% (v/v) methacryloxypropyl–trimethoxysilane solution in methanol. Afterwards a degassed solution of 4% (w/v) T acrylamide in 0.1 *M* Tris–boric acid (2 m*M* EDTA) pH 8.2 containing 5 μl of a 10% (w/v) ammonium persul-

Table 1
Characteristics of the cyclodextrins used

Type of cyclodextrin	Degree of derivatisation (molecules per CD ring)	Average M_r (g/mol)	Average formula
Hydroxypropylated β-CD	6.3	ca. 1500	$C_{61}H_{108}O_{41}$
Heptakis (di-O-methyl) β-CD	14	1331	$C_{56}H_{98}O_{35}$
Carboxymethylated β-CD	3.6	ca. 1340	$C_{49}H_{77}O_{42}$
Carboxymethyl β-CD-polymer		ca. 8700	$C_{49}H_{77}O_{42}$
Carboxyethylated β-CD	ca. 6	ca. 1570	$C_{60}H_{94}O_{47}$
Succinylated β-CD	3.5	ca. 1490	$C_{56}H_{84}O_{46}$

fate solution and 5 μl of a N,N,N′,N′-tetraethylmethylendiamine solution per ml acrylamide solution was filled into the capillary. After polymerisation the formed gel inside the column was pressed out leaving a coating at the inner capillary wall. As long as buffers up to pH 7 were used this coating was stable for several months. The stability of the coating was determined by the absence of a significant EOF. The CD derivatives employed in this paper are summarized in Table 1. The data with the degree of modification were obtained from the manufacturer. The hydroxypropyl CDs were obtained from Wacker Chemie (Germany). All other CDs were bought from Cyclolab (Budapest, Hungary). The enantiomers used (Fig. 1) were kind gifts of Professor Knabe and Dr. Dorfmüller from the Pharmaceutical Department and of Dr. Maurer from the Clinical Department of our university. Deionised water from a Milli-Q system (Millipore) was used for buffer preparation. Chemicals such as spermine tetrahydrochloride $[H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2 \cdot 4HCl]$, spermidine trihydrochloride $[H_2N(CH_2)_4NH(CH_2)NH2 \cdot 3HCl]$ and CTAB were obtained from different suppliers and were all of analytical grade. The experiments were performed on a P/ACE system 2000 (Beckman Instruments, Fullerton USA) and a HP^{3D} CE system (Hewlett-Packard, Waldbronn, Germany).

3. Results and discussion

The possibility to detect cations as well as anions in one electrophoretic run is due to the presence of a strong EOF in an untreated fused-silica capillary. Since a regular EOF is directed towards the cathode all cations are detected in front of the EOF (the fastest ions first) while the anions migrating slower than the EOF are detected behind the EOF (the fastest anions last) in an electropherogram. This means that reversing the EOF, and also reversing the polarity of the electrodes should result in a reversed migration pattern of the analyte, i.e. the fastest anions are first and the fastest cations are detected last in an electropherogram. Long-chained tetraalkylammonium compounds with a chain length of C_{10} to C_{20} are well known in CE as additives for the reversal of the EOF. CTAB was tested here as a potential flow reversal agent together with β- and hydroxypropyl β-CD. As demonstrated in Fig. 2 CTAB is not able to change the direction of the EOF when β-CDs and their derivatives are present in the running buffer. A similar behaviour was found by Quang and Khaledi [29] for basic cationic enantiomers at low pH values using β-CD and CTAB. In our case anions were separated at high pH values (pH 8.3). Fig. 2A shows the separation obtained without any CTAB added to the buffer. All anions migrate behind the EOF and were separated using hydroxypropyl β-CD. The same buffer system with a concentration of 0.5 m*M* CTAB, which is a very suitable concentration for an EOF reversal, does not give the expected result. The EOF is still directed towards the cathode. Increasing the CTAB concentration results only in a decrease of the velocity of the EOF and therefore longer analysis time. Without any cyclodextrin in the buffer, 0.5 m*M* CTAB is sufficient to revers the EOF and all analytes are detected in the reversed migration order (not

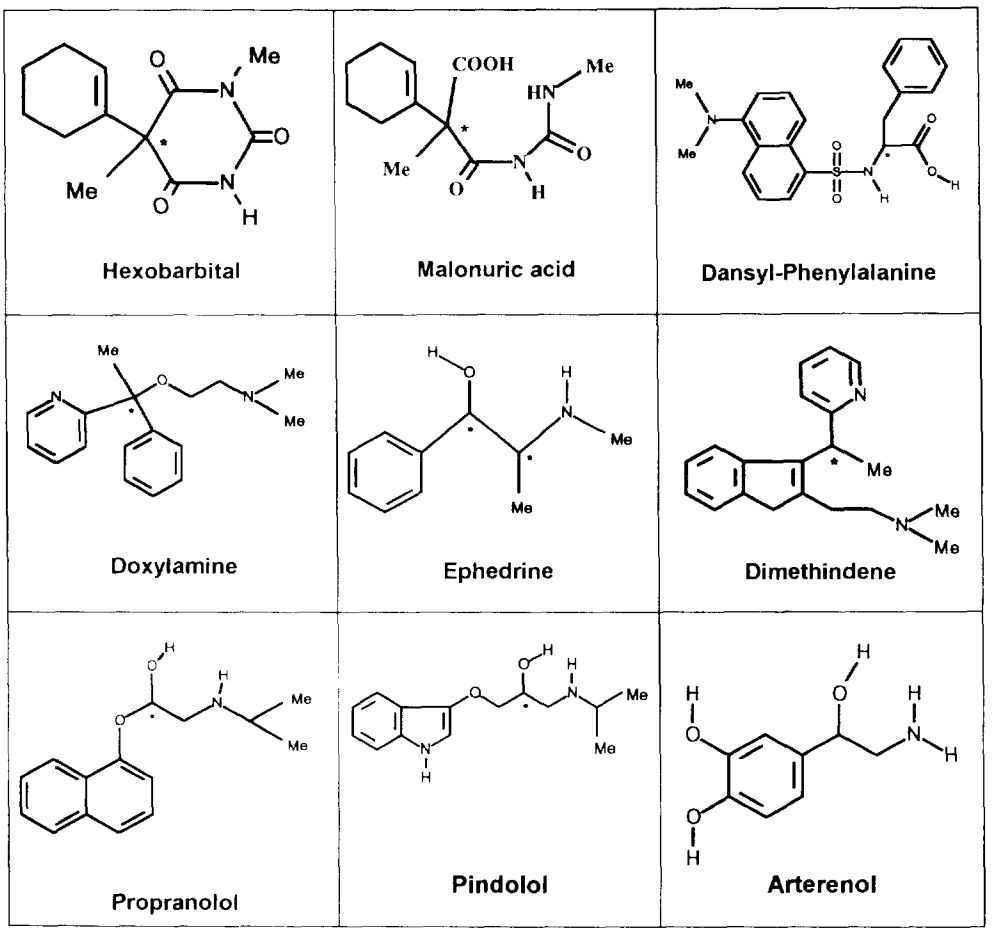

Fig. 1. Structures of the samples.

shown here) but without chiral resolution because no chiral selector is added. Even at a concentration of 1 m*M* CTAB which is above the critical micellar concentration (CMC) of CTAB the EOF is still not reversed (Fig. 2C). The faster migrating analytes [last in an electropherogram (peak 3)] are no longer detected in the given time because the EOF is too slow under these conditions. This clearly indicates that there is a mechanism preventing the CTAB from forming a double layer at the inner surface of the capillary wall. This can be explained by the inclusion of the long alkyl chain of CTAB into the cavity of the CD which results in a lower effective concentration in free solution and therefore increasing the CMC of the detergent and also hindering the formation of the double layer at the capillary wall.

Peak 3 in Fig. 2A and B represents a degradation product of hexobarbital (peak 1) which comes from alkaline hydrolysis of this urea derivative. During hydrolysis of (−)-hexobarbital the optical rotation is changing from the (−)-form to (+)-malonuric acid [30]. Although the steric configuration around the asymmetric carbon atom stays the same during the hydrolysis the complexing properties for the hydrolysis product to the CD have changed dramatically. Ring hydrolysis of the (−)-hexobarbital and the generation of additional functional groups with high hydrogen-bonding capabilities (carboxylic groups and amid groups) result in stronger bind-

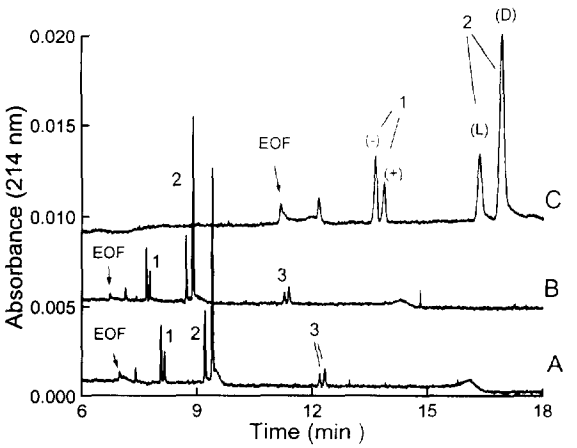

Fig. 2. Influence of CTAB as an EOF flow modifier on the migration direction of the analyte. Run conditions: 67 cm (60 cm to detector) × 75 μm I.D. uncoated capillary, 370 V/cm, 100 m*M* Tris–boric acid pH 8.35. Sample: hexobarbital spiked with (−)-hexobarbital (1), dansyl-phenylalanine spiked with D-dansyl-phenylalanine (2), hydrolysis product of hexobarbital (3), detection at the cathodic end (cathodic EOF in all cases). Instrument: P/ACE 2000. (A) buffer contains 1% (w/v) hydroxypropyl β-CD without CTAB; (B) as (A) but with 0.5 m*M* CTAB; (C) as (A) but with 1 m*M* CTAB.

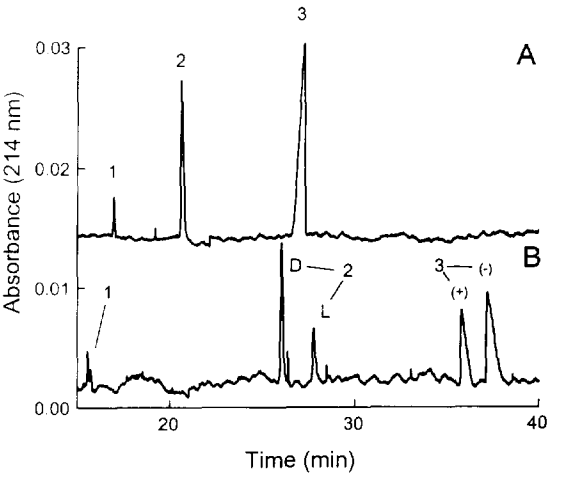

Fig. 3. Influence of spermidine as a flow modifier on the migration direction of the analytes. Run conditions: 67 cm (60 cm to detector) × 75 μm I.D. capillary, 25°C, buffer: 100 m*M* Tris, 100 m*M* boric acid pH 8.3 containing 5 m*M* spermidine hydrochloride, detection at 214 nm. Sample: hydrolysis product of hexobarbital (1) (from alkaline hydrolysis of hexobarbital), dansyl-phenylalanine spiked with D-dansyl-phenylalanine (2), hexobarbital spiked with (−)-hexobarbital (3). Instrument: P/ACE 2000. (A) Applied voltage: 20 kV, no cyclodextrin added to the buffer, detection at the anodic end (anodic EOF); (B) applied voltage: 25 kV, 0.7% (w/v) of hydroxypropyl β-CD added to the buffer, detection at the anodic end (anodic EOF).

ing of the (+)-malonuric acid to the CD. This also demonstrates that small changes in the spacial configuration away from the chiral center as well as the rotation freedom around the same asymmetric carbon atom can have a very strong effect on the stability of the CD–analyte complex. As a result the separation of this analyte may stay the same, or being lost, or even reversed although the absolute configuration on the chiral center stays the same.

Employing a concentration of 5 m*M* spermidine in the running buffer without CD added, the EOF as well as the migration order of the analyte could be reversed (towards the anode). This is shown in Fig. 3A. In Fig. 3B, hydroxypropyl β-CD was added to the same buffer as used in Fig. 3A. It can be seen that enantiomeric separation is achieved and the migration order of all enantiomers is reversed compared to the electropherogram shown in Fig. 2A. Although the buffers were filtered and all reagents were of analytical grade the baseline was found increasingly noisy with analysis time. This baseline noise could be reduced by exchanging spermidine with spermine as a flow modifier in the running buffer. Fig. 4A shows the EOF in direction to the cathode without spermine while in Fig. 4B the EOF is oriented towards the anode after addition of this amine to the buffer. The migration order of the enantiomers has been changed under these conditions; however, including the appearance of the EOF, analysis time has doubled. The advantages of the flow reversal in CE can be seen in Fig. 5. It shows the separation of impurities of hexobarbital with (left electropherogram) and without (right electropherogram) reversal of the EOF. It is shown that the migration order of all analytes is reversed when reversing the EOF. Beside the hydrolysis products other impurities which may be residues from the manufacturing process are also detectable. The sum of peak areas of these impurities are in the 0.1% range compared to the main peaks. Employing the regular mode

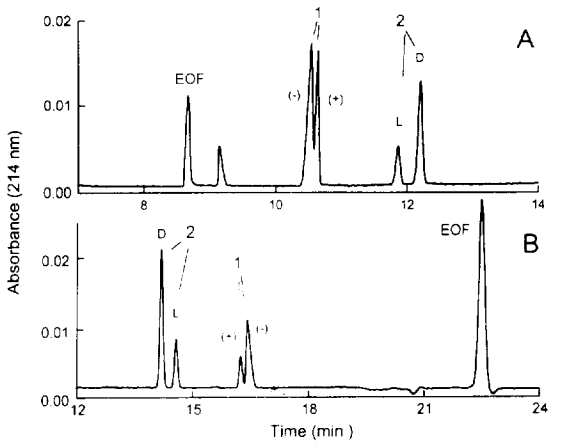

Fig. 4. Influence of spermine as a flow modifier on the migration direction of the analytes. Run conditions: 67 cm (60 cm to detector) × 75 μm I.D. capillary, 25°C, applied voltage: 20 kV, buffer: 100 m*M* Tris, 100 m*M* boric acid pH 8.3 containing 5 m*M* spermine hydrochloride, 0.7% (w/v) of hydroxypropyl β-CD added to the buffer, detection at 214 nm. Sample: hexobarbital spiked with (−)-hexobarbital (1), D,L-dansyl-phenylalanine spiked with D-dansyl-phenylalanine (2). Instrument: P/ACE 2000. (A) Detection at the cathodic end (cathodic EOF), no EOF flow modifier added; (B) buffer containing 5 m*M* spermine hydrochloride, detection at the anodic end (anodic EOF).

[anodic EOF (Fig. 5 on the right)] a further increase of the injected amount leads to distortion of all minor components and the components labelled as a, b, c comigrate with the main peak and cannot be analysed any more which would of course be possible if they are migrating in front of the main components.

Because the EOF has a very low anodic mobility when spermine and spermidine are employed, the analysis of fast moving cationic enantiomers cannot be achieved. For these analytes a very fast anodic EOF with the same velocity as the regular EOF would be appropriate. Therefore, in the following other methods are described for the reversal of the migration order. A mixture of 6 basic aromatic enantiomeric drugs have been used here to demonstrate and examine the separation capabilities of the different CDs as well as their selectivities in terms of changing the migration order. Fig. 6 shows the separation of the test mixture employing heptakis(2,6-di-O-methyl)-β-CD which is a widely used chiral selector so far. This selector was able to resolve four out of the six compounds. The (+)-ephedrine isomer was found

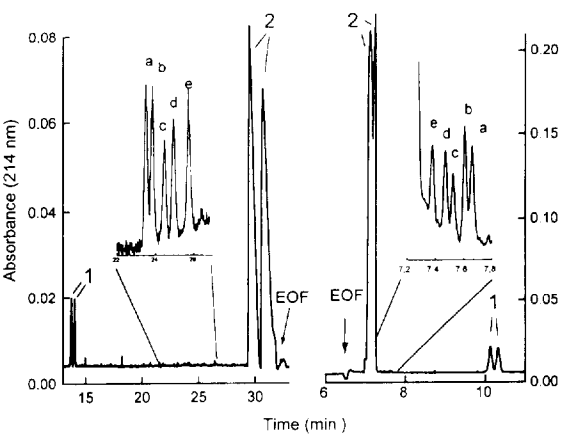

Fig. 5. Detection of impurities with cathodic and anodic electroosmotic flow. Run conditions: 57 cm (50 cm to detector) × 75 μm I.D. uncoated capillary, detection at 214 nm. Peaks: 1 = (±) hydrolysis product; 2 = (±)-hexobarbital; a, b, c, d, e = impuities. Instrument: P/ACE 2000. (A) 430 V/cm, buffer: 0.1 *M* TBE (Tris–boric acid–EDTA) pH 8.3, 1.56% (w/v) β-CD, cathodic EOF; (B) 170 V/cm, buffer: 0.1 *M* TBE pH 8.3, 1.56% (w/v) β-CD, 5 m*M* spermine · HCl, reversed polarity, anodic EOF.

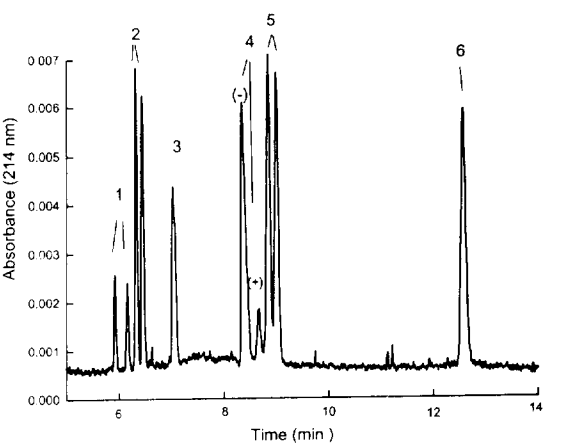

Fig. 6. Separation of basic amines using heptakis(di-O-methyl)-β-CD. Run conditions: 27 cm (20 cm to detector) × 75 μm I.D. coated capillary (linear polyacrylamide), 370 V/cm, 20 m*M* phosphate buffer pH 2.2 (adjusted with HCl and Tris), 30 m*M* heptakis(di-O-methyl)-β-CD. Instrument: P/ACE 2000. Peaks: 1 = arterenol; 2 = doxylamine; 3 = dimethindene; 4 = ephedrine, spiked with (−)-ephedrine; 5 = pindolol; 6 = propranolol.

to migrate behind the (−)-enantiomer [31]. Under the same experimental conditions the determination of optical impurities is hardly possible because the efficiencies in CE strongly depend on the sample loading of the column and the selectivity is not high enough to compensate for this. Therefore resolution is decreasing when higher sample amounts are injected. Employing the carboxymethylated β-CD, baseline resolution of all enantiomers could be achieved with much higher sample load (Fig. 7). Surprisingly, with this chiral selector the (+)-enantiomer of ephedrine migrates faster. This shows clearly that the affinity of enantiomers to CDs strongly depends on the type and degree of derivatisation on the CD. Also there is no need to reverse the migration order with CDs built of D-(−)-glucose instead of D-(+)-glucose units.

As discussed above trace analysis of optical impurities should be done in front of the main component especially when wall effects are observed. Since this is the case for (+)-ephedrine using the carboxymethylated β-CD, the overloading capability and the detection limit was studied for this compound in this buffer system. Furthermore a short coated column was used here to maintain high electric field strength, to speed up the analysis and reduce wall adsorption for these basic analytes [32]. As can be seen in Fig. 8 the detection of 0.3% of the (+)-ephedrine was possible beside the main component. Applying a longer capillary and therefore increased resolution should allow to increase the overloading of the column and achieve higher signals for both the main component and its optical impurity. By doing this the buffer capacity must be consequently increased to minimize electrodispersion due to high conductivity in the sample zones. In the reversal experiment the determination of the (−)-ephedrine in excess of the (+)-ephedrine was only possible in the 1–2% range under identical run conditions. Also a capillary with a three times extended light path at the detection point (75 μm I.D., 225 μm I.D.

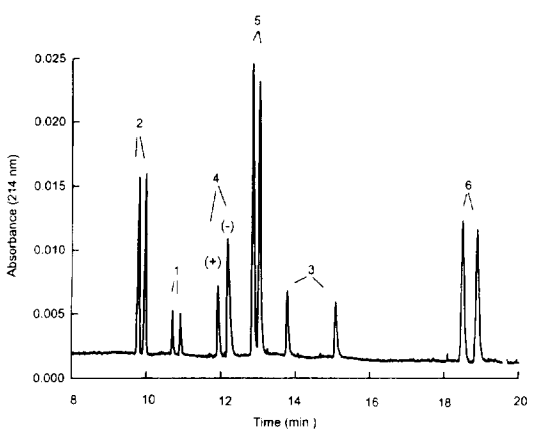

Fig. 7. Separation of basic amines using carboxymethyl β-CD. Run conditions: 67 cm (60 cm to detector) × 75 μm I.D. capillary, 340 V/cm, 20°C, 20 m*M* citric acid pH 2.5 containing 2% (w/v) of the CD. Instrument: P/ACE 2000, racemic mixtures of: doxylamine (2), arterenol (1), ephedrine (4) spiked with (−)-ephedrine, pindolol (5), dimethindene (3) and propranolol (6).

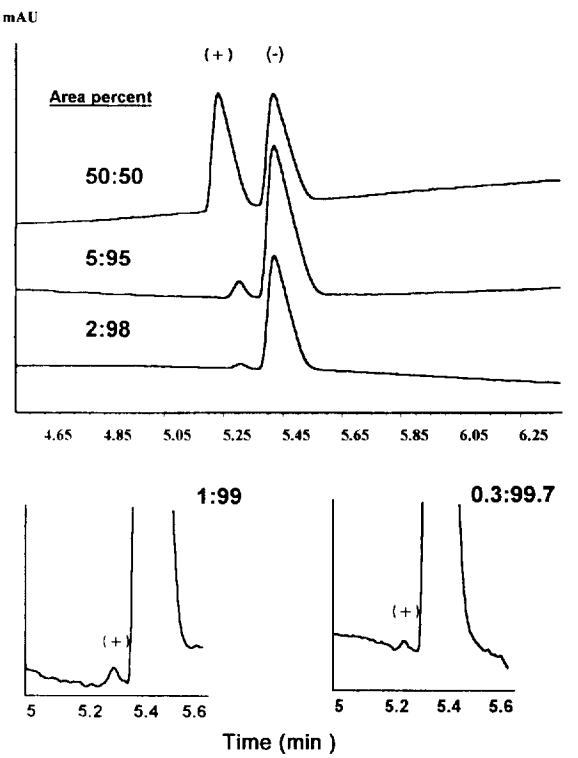

Fig. 8. Determination of the enantiomeric excess of (−)-ephedrine. Run conditions: 32.5 cm (32 cm to detector) × 75 μm I.D. fused-silica capillary with three times extended light path, 470 V/cm, capillary coated with 4% T linear polyacrylamide, pressure injection 10 mbar for 3 s each, 20 m*M* citric acid buffer pH 2.5 containing 2% (w/v) carboxymethylated β-CD. Instrument: HP^{3D} system.

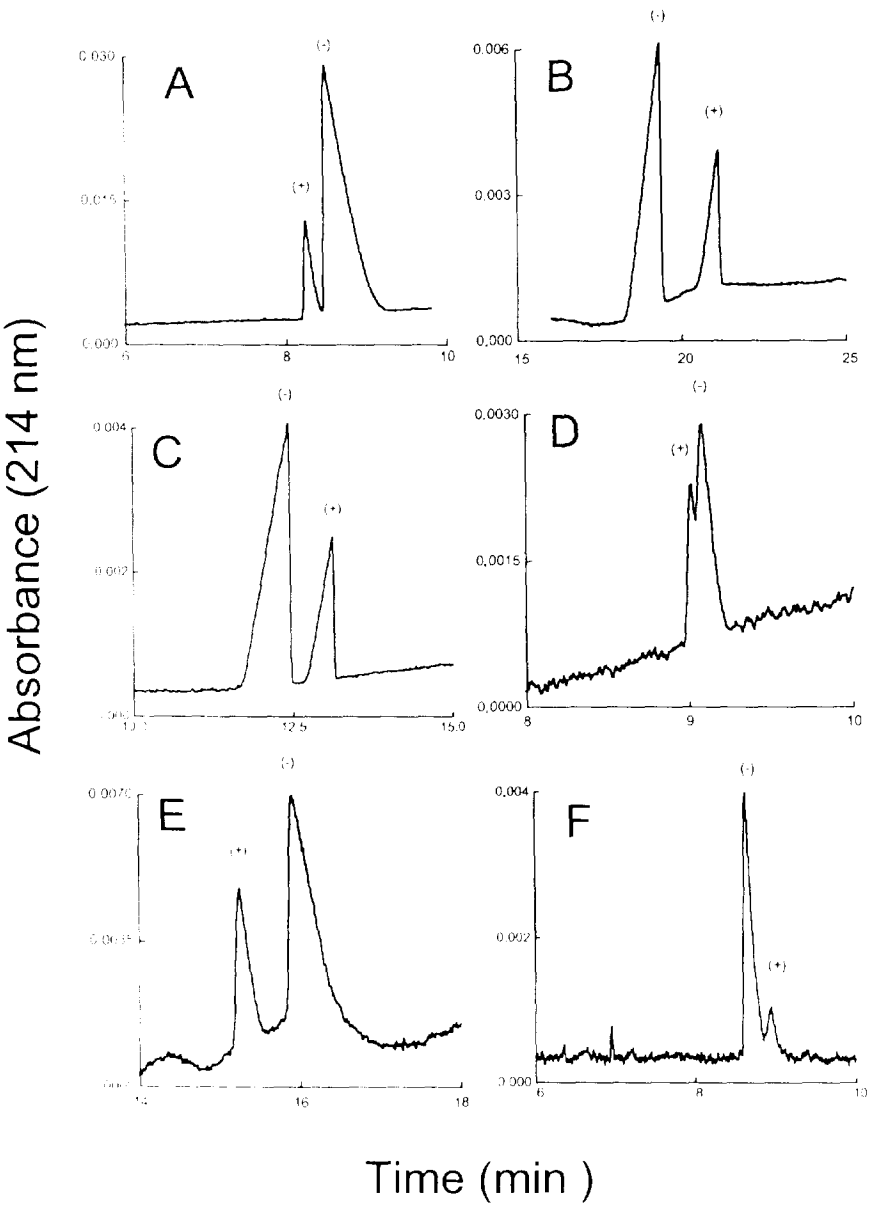

Fig. 9. Reversal of the migration order of enantiomers with pH. Run conditions: sample: ephedrine spiked with (−)-ephedrine, Instrument: P/ACE 2000, detection at 214 nm. (A) 27 cm (20 cm to detector) × 75 μm I.D. coated capillary, 370 V/cm, 25°C, buffer: 20 m*M* citric acid pH 2.8 containing 2% (w/v) carboxymethyl β-CD, detection at the kathodic end. (B) 37 cm (30 cm to detector) × 75 μm I.D. coated capillary, 270 V/vm, 20°C, buffer: 1.5% (w/v) carboxymethyl β-cyclodextrin adjusted to pH 7.2 with Tris, detection at the anodic end. (C) 27 cm (20 cm to detector) × 75 μm I.D. coated capillary, 370 V/cm, 20°C, buffer: 1% (w/v) carboxyethyl β-cyclodextrin adjusted to pH 6.2 with Tris, detection at the anodic end. (D) 37 cm (30 cm to detector) × 75 μm I.D. coated capillary, 540 V/cm, 25°C, buffer: 20 m*M* citric acid pH 2.2 containing 1.55% (w/v) succinyl β-cyclodextrin, detection at the cathodic end. (E) 37 cm (30 cm to detector) × 75 μm I.D. coated capillary, 270 V/cm, 25°C, buffer: 1% (w/v) succinyl β-cyclodextrin adjusted to pH 5.7 with Tris, detection at the cathodic end. (F) 27 cm (20 cm to detector) × 75 μm I.D. coated capillary, 370 V/cm, 25°C, buffer: 20 m*M* phosphate buffer pH 2.2 (adjusted with Tris and HCl) containing 2% (w/v) heptakis(2,6-di-O-methyl)-β-cyclodextrin, detection at the cathodic end.

at the detection point ("bubble cell") was used in this experiment giving a 3–5 times better signal-to-noise ratio compared to a non-expanded capillary.

Another way for reversal of the migration order is the use of charged CDs, where the charge can be altered by changing pH. CDs with carboxylic functions are deprotonated at pH values above pH 5 and the charge of the carboxylic group leads to an own mobility of the CDs. On the other hand at pH values below 3 the CD is no longer charged and behaves like a regular CD. At these pH values the enantiomer with the highest affinity to the chiral selector will be retarded longer and thus will show the slowest mobility. In the charged mode at higher pH values the CD can act as a carrier for the analytes with opposite charges and the stronger binding enantiomer will be detected first. These effects can be observed only if the introduced charge on the CD ring does not affect the enantiomeric selectivity. Also a higher number of charges are necessary on the CD to compensate for the analyte charges because neutral CD complexes are not transported to the detector. This would implicate a kind of ion-pairing mechanism. The strength of interaction of the solute with the CD is then responsible for the migration direction of the enantiomers.

Table 2 summarizes the possibilities of separation and the migration order of ephedrine with different CDs as chiral selectors at different pH values. All charged CDs show a different selectivity dependence on the pH value. Beside β-CD-phosphate (which is always charged) and heptakis(2,6-di-O-methyl)-β-CD (which is not charged at usual pH values) all other CDs show dramatic changes when going from the charged to the uncharged mode of these CDs. Carboxymethyl β-CD was able to resolve (±)-ephedrine as well in the uncharged (below pH 3) as in the charged mode (above pH 5). The reversal of the migration order can be explained as already discussed. Carboxyethyl β-CD is not able to resolve this solute under acidic conditions (pH 2.5) in the uncharged mode. At pH values above 5 the anionic carboxyethyl β-CD groups can act as a carrier (like carboxymethyl β-CD) and transports the enantiomeric cations to the anode, where the detector has to be placed. Only in the charged mode this CD allows the separation of the enantiomers. The succinylated β-CD also shows resolution for ephedrine in the charged and in the uncharged mode. Although higher concentrations had to be used at low pH values in order to obtain resolution, in the charged mode higher resolutions are achieved with lower CD concentrations. Because the binding of the analyte is not strong enough, the anionic CD does not form ion pairs and cannot act as a carrier for the cationic enantiomers. The migration direction of the ephedrine is here still towards the cathode. However the (−)-ephedrine migrates first with this CD. Using the phosphated β-CD and the carboxymethyl β-CD polymer no resolution could be observed for

Table 2
Resolution and migration order of (+)-ephedrine with different cyclodextrins

Cyclodextrin	Resolution (low pH) (uncharged mode)	Resolution (high pH) (charged mode)	Migration order reversal
Carboxymethyl β-CD	(+) First[a]	(−) First[a]	Yes
Carboxyethyl β-CD	No resolution	(−) First[a]	No
Succinyl β-CD	(+) First[a]	(+) First[a]	No
Carboxymethyl β-CD-polymer	No resolution	No resolution	No
β-CD Phosphate	Always charged	No resolution	No
Heptakis(2,6-di-O-methyl)-β-CD	(−) First[a]	Not charged	No

[a] Coated column (suppressed EOF) and polarity must be reversed when going from the charged to the uncharged mode of the cyclodextrin.

ephedrine neither at low nor at high pH values. Triangular peaks were a result of high mass load and therefore overloading of the buffer capacity.

In this study (±)-ephedrine was used as a test compound. This implicates that for other solutes the migration order reversal may be observed using other CDs than the carboxymethylated β-CD.

4. Conclusions

It has been demonstrated that detergents with quaternary ammonium groups are not suitable for reversal of the EOF when β-CD derivatives are present as chiral selector in the running buffer. Polyamines were found to be very suitable for this purpose although the mobility of the EOF is very slow towards the anode. This hinders their application in the reversal of the migration order of cationic enantiomers as well. It is demonstrated that the analysis of impurities of anionic enantiomers can be achieved when overloading the column.

Since a slow reversed EOF cannot be used for the separation of fast cationic enantiomers other methods have been described here in order to achieve a reversal of the migration order. Different derivatized CDs are potential candidates for this goal. A very elegant method is the use of chargeable CDs which can be ionized depending on the pH used in the running buffer. With these CDs a change in pH can reverse the migration order as well. As demonstrated with ephedrine, the determination of the optical impurity in the 0.1% range is possible in CE when the minor compound is migrating in front of the main component.

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