Selected applications of capillaries with dynamic or permanent anodal electroosmotic flow in chiral separations by capillary electrophoresis[1]

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Abstract

The techniques for a dynamic and permanent reversal of the electroosmotic flow (EOF) were used for the reversal of the enantiomer migration order (EMO) of neutral and cationic analytes in chiral capillary electrophoresis (CE). Native β-CD and an anionic CD derivative, CM-β-CD were used in both, bare silica- and positively coated capillaries. Advantages and disadvantages of a dynamic and permanent modification of the capillary inner surface are briefly discussed. © 1997 Published by Elsevier Science B.V.

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1. Introduction

The electroosmotic flow (EOF) is one of the most important factors affecting resolution of sample components in capillary zone electrophoresis (CZE). As generally accepted, the EOF is a non-separative transport in contrast with electrophoretic mobility of analytes (μ_{el}) which should be separative in order to achieve the resolution of sample components.

A minimization of a contribution of non-separative transport (i.e., the mobility of the EOF, μ_{EOF}) in the overall mobilities of the analytes favors a higher separation factor (α). This is basically true for achiral separations which are actually based on the electrophoretic separation principle, e.g., on a different mobility of analytes depending on their charge density. However, chiral separations in CE conceptually do not belong to this category. Neither intrinsic electrophoretic mobility (μ_{el}) nor the EOF (μ_{EOF}) are separative for the enantiomers in an achiral medium and both of them can become separative with equal success in a chiral medium. Strictly to say, a separation of the enantiomeric pair is conceptually impossible in a free solution CZE and becomes possible only after introduction into the

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separation system of an additional selectivity factor which differs from the electrophoretic one. These are stereoselective selector-selectand interactions in chiral CZE.

Although several studies in chiral CE separations illustrate the improvement of separations in capillaries without the EOF [1,2]. This effect is basically achieved by the modification of the migration times and solute-capillary wall interactions and not as a result of supression of 'nonseparative' contribution of the EOF. Therefore, performing a separation of the enantiomers in coated capillaries with a suppressed or totally eliminated EOF seems to be favorable. Several studies on the CE enantioseparation in coated capillaries confirm this [1,2].

The EOF can be sometimes very desirable in CE [3,4]. The most important advantage of the EOF is that it is not a dispersive flow in contrast to the pressure-driven laminar flow [5,6]. Due to this reason the EOF does not disturb a zone distribution in a separation capillary and additionally, it allows the simultaneous separation of anionic, cationic and neutral analytes [3,7]. The EOF can be used also for a desirable adjustment of the analysis time. When the direction of μ_{EOF} and μ_{el} coincide the EOF can result in a shorter analysis time and sharper zones (high peak efficiency N). In contrast to this, when μ_{EOF} and μ_{el} are oppositely directed the EOF results in prolonged sample residence time in a separation capillary which may favor a higher separation factor (α). The use of cathodal EOF for the enantioseparation of neutral analytes [8–12] and for the reversal of the enantiomer migration order (EMO) [4] were reported recently.

The principal advantages of the capillaries with positively coated inner surface are the following: (a) suppression of interaction between positively charged analytes and capillary inner wall and (b) anodal EOF. The former is more important in the analysis of high-molecular-weight compounds, whereas the latter can be equally useful in the analysis of high- as well as low-molecular-weight compounds.

The favorable effect of anodal EOF on the CE-enantioseparations of cationic analytes has been reported [13,14]. Moreover, the reversal of the EMO of charged chiral analytes as a result of a dynamic reversal of the EOF has been described [15,16].

Two different types of the reversal of EMO of neutral and cationic analytes is discussed in the present study. Both types of the reversal of EMO are based on the employment of anodal EOF. The fundamental aspects of the reversal of the EMO in chiral CE are also briefly discussed.

2. Selected fundamental aspects

The simplified mobility difference equation was derived for the separation of ions in slab-gel electrophoresis in 1969 [17]. A Similar equation for the separation of the enantiomers in CE was derived by Wren and Rowe [18]:

$$\Delta\mu_{(R-S)} = \frac{(\mu_0 - \mu_1)(K_R - K_S)[C]}{1 + [C](K_R + K_S) + K_R K_S[C]^2} \quad (1)$$

where $\Delta\mu_{(R-S)}$ is the mobility difference between the R and S enantiomers of a given chiral analyte, μ_0 and μ_1 are the mobilities of the free and the complexed analytes, respectively, K_R and K_S are the binding constants of the R and the S enantiomers to the chiral selector, respectively, and $[C]$ is the concentration of a chiral selector.

In contrast to pressure-driven separation techniques the self-mobility of an analyte in CE can be directed to the detector as well in the opposite direction. Therefore, it is important to consider the vector properties of a mobility in CE and to rewrite the Eq. (1) in the vectorial form [19]:

$$\Delta\vec{\mu}_{(R-S)} = \frac{(\vec{\mu}_0 - \vec{\mu}_1)(K_R - K_S)[C]}{1 + [C](K_R + K_S) + K_R K_S[C]^2} \quad (2)$$

This equation allows the consideration of almost all principal possibilities for the reversal of EMO in chiral CE except one, very rear, case. The last can be obtained by analyzing a more complex form of Eq. (2):

$$\Delta\vec{\mu}_{n(R-S)} = \frac{\vec{\mu}_0 + \vec{\mu}_1 K_{R,x}[C]}{1 + K_{R,x}[C]} - \frac{\vec{\mu}_0 + \vec{\mu}_2 K_{s,x}[C]}{1 + K_{s,x}[C]} \quad (3)$$

The Eq. (3) is the same as derived by Wren et al. [20] but written in vectorial form.

The reversal of EMO means that the vector $\Delta\bar\mu_{(R-S)}$ changes the sign. This is equivalent to the change of its direction. As Eq. (2) shows following possibilities exist for the reversal of the sign of $\Delta\bar\mu_{(R-S)}$:

(a) $K_R > K_S$ changes for $K_R < K_S$ (only this possibility exists for the reversal of elution order of the enantiomers in pressure-driven separation systems).

(b) $\mu_0 > \mu_1$ changes for $\mu_0 < \mu_1$.

(c) Neither (a) nor (b) occurs but the vector $\Delta\bar\mu_{(R-S)}$ is reverted by applying an additional migration force to a separation system.

The last case is not obvious and needs to be clarified. As shown in Fig. 1, $\Delta\bar\mu_{(R-S)}$ should be directed to the detector in order to achieve a detection of a sample. If an additional migration force will be applied to the separation system which will be higher in magnitude than $\Delta\bar\mu_{(R-S)}$ and directed oppositely to it, than the detector should be replaced to the opposite end of the separation capillary in order to achieve a detection of a sample. This operation can be imitated in CE by changing the polarity of the high voltage supply. As shown in this schema, after reverting
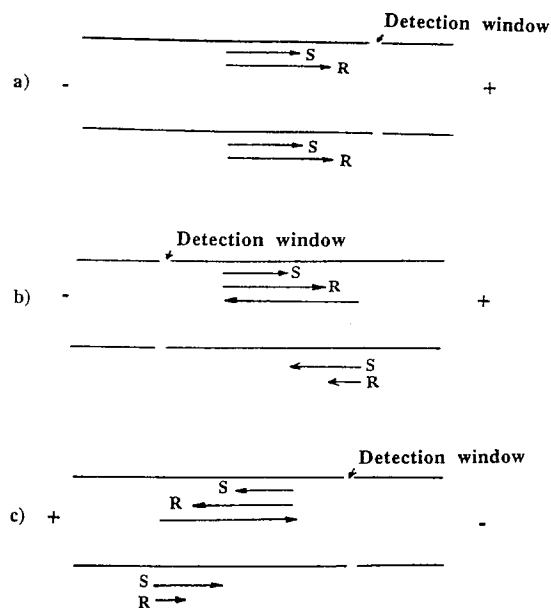
of the vector $\Delta\bar\mu_{(R-S)}$ the relation between the intrinsic mobilities of the R and the S enantiomers remains the same, but the relation between their net mobilities changes for the opposite one. Thus, if for a given selector-selectand pair, one can interchange the polarities of the injection and the detection end of a capillary, than the reversal of the EMO can be observed without any changes in the selector/selectand binding and/or mobilities.

Another important point which should be mentioned is that the EOF does not enter Eq. (1) Eq. (2) Eq. (3). This means that the EOF cannot affect the intrinsic migration order of the enantiomers. However, the EOF can act as a additional driving force as mentioned above which allows to revert the vector $\Delta\bar\mu_{(R-S)}$. Thus, the EOF allows to observe the apparent reversal of the EMO. Only the case (c), i.e. the apparent reversal of the EMO, is the subject of this study.

3. Experimental

3.1. Reagents and racemic compounds

Mesityl oxide, spermine tetrahydrochloride [N,N'-bis-(3-aminopropyl)-1,4-diaminobutan tetrahydrochloride], polyethylenimine polymer (M_r 600 000–1000 000) (PEI) were from Fluka (Buchs, Switzerland). Sodium hydroxide, phosphoric acid, sodium dihydrogen phosphate, acetic acid, succinic acid and citric acid were from Merck (Darmstadt, Germany).

Racemic thalidomide and optically pure R(−) and S(+) enantiomers of thalidomide were gift from Pharma Grünenthal AG (Grünenthal, Germany). Racemic benzoin and 5-methyl-5-phenylhydantoin were from Aldrich (Steinheim, Germany) and resolved to enantiomers in our laboratory using analytical scale cellulose-phenyl-carbamate-type chiral stationary phase [21]. Racemic dimethindene maleate was a gift from Zyma (Munich, Germany). R(−) and S(+) enantiomers of dimethindene were obtained in our laboratory using diastereomeric crystallization with optically pure tartaric acid in ethanol as described in [22].
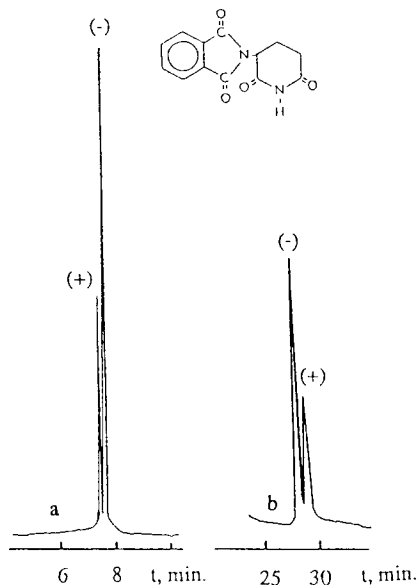

Fig. 1. Schematic representation of the apparent reversal of EMO.

Fig. 2. Reversal of the migration order of [R(−): S(+) = 2:1] mixture of thalidomide enantiomers in absence (a) and presence (b) of 7 mM spermine hydrochloride in the background electrolyte. Conditions: 50 mM phosphate buffer (pH 4.5), without spermine hydrochloride (case a) and the same buffer containing 7 mM spermine hydrochloride (case b). Field strength 400 V cm⁻¹, injection at the anode (a) and at the cathode (b). CM-β-CD concentrations were 5 mM and 10 mM in cases (a) and (b), respectively. Bare silica capillary (50 μm) with 60 cm total length and 43 cm effective length.

β-cyclodextrin (β-CD) and carboxymethyl-β-CD (CM-β-CD) with substitution degree approx. 2.1 were gift from Wacker Chemie (Munich, Germany).

3.2. Capillaries

Untreated fused-silica capillary (60 cm total length, 43 cm effective length, 50 μm i.d.) was supplied from Grom (Herrenberg, Germany).

A capillary with permanently positively charged inner surface was prepared by physical adsorption of PEI on the fused-silica capillary inner surface as described in [23]. The fused-silica capillary was treated with a solution of 1 M sodium hydroxide for 30 min and with bi-distilled water for 15 min. Then the capillary was flushed with a solution of PEI in water for 10 min and PEI solution was left in the capillary for 1 h. Then the polymer-solution

was extruded out of the capillary by rinsing with water for 15 min and with running buffer for 15 min. This capillary was used for the enantioseparation without further treatment.

3.3. Equipment

A Grom capillary electrophoresis system 100 (Grom, Herrenberg, Germany) equipped with a Linear Instruments (Reno, NV, USA) UVIS 200 detector and an HP 3396 A integrator (Hewlett-Packard), Avondale, PA, USA) was used. The racemic samples were introduced hydrostatically (10 cm). The electric field was 400 or 500 V cm⁻¹. The anode and cathode buffers had the same pH and molarity as the run buffer.
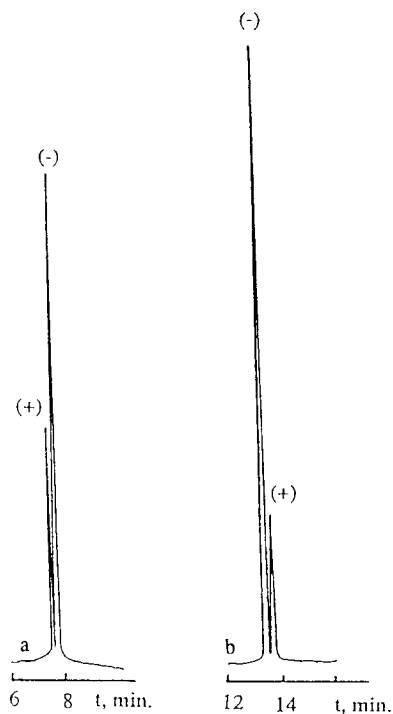

Fig. 3. Reversal of the migration order of [R(−): S(+) = 2:1] mixture of thalidomide enantiomers in bare silica capillary (a) and in capillary with positively charged inner surface (b). Other conditions for cases (a) and (b) were the same as in Fig. 2.

3.4. Procedures

The enantioseparations were performed in buffers which are specified in the legends for figures. The pH was adjusted using 50 mM sodium hydroxide or 50 mM phosphoric acid solutions. β-CD and CM-β-CD were used as chiral selectors in the concentrations specified in the legends. The detection was performed at 210 nm. Mesityl oxide was used as a neutral marker for the measurement of the electroosmotic flow (EOF). The separation selectivity (α) peak efficiency (N) and the resolution factor (R_s) were used in order to characterize the separation. These characteristics were calculated following the equations commonly used in CE.

4. Results and discussions

4.1. Reversal of EMO of neutral chiral analytes

A reversal of EMO is desirable sometimes for a better quantification of a minor component in a nonracemic mixture of the enantiomers [16,24]. Additionally, this technique can be very useful for the peak identification [25,26] and in mechanistic studies.

Several types of the reversal of EMO are possible in CE as summarized in the recent review on this subject [19].

Two principal driving-forces for the neutral analytes in CE are the EOF and a carrier ability of a charged chiral selector [27]. Based on this following possibilities exist for a reversal of the EMO of neutral analytes in CE:

(a) opposite chiral recognition ability of chiral selectors;

(b) alternative use of the EOF and oppositely directed mobility of a chiral selector as driving forces for the neutral analytes. The inner surface of the separation capillary and the chiral selector should have the same charge to make feasible this type of reversal of EMO.

(c) alternative use of the oppositely directed EOFs in a bare fused-silica capillary and that one with positively charged inner surface.

The first technique (a) is well suitable for the chiral selectors which are available in both configurations [28]. The chiral recognition abilities of differently derivatized CDs can be opposite, in principle [19], however this is rather exception than the common case because all CDs are build from D-glucose units and no optical antipodes are available for CDs.

The second technique (b) requires the use of charged CDs with high substitution degree with strong acidic or basic functionality's in order to warrant high self-mobility together with tight binding with analytes which are necessary preconditions for a carrier ability of a chiral selector.

The third technique for the reversal of the EMO does not necessarily require a high carrier ability of a charged chiral selector and can be well designed.

There are two principal possibilities in CE for a reversal of the EOF: (a) dynamic and (b) permanent modification of the capillary inner wall with positively charged compounds. The advantage of a dynamic modification is that the same capillary can be used for a 'direct' and 'reversed' migration of the analyte enantiomers just changing the buffer (without or with cationic surfactant, respectively). The possible interaction of EOF modifiers with a chiral selector and a relatively low anodal EOF is mentioned in literature as the disadvantages of this technique [16].

The combination of charged CD (in this case negatively charged CM-β-CD) and a short alkyl-chain cationic surfactant spermine tetrahydrochloride allows the reversal of the EMO of a neutral chiral compound thalidomide (Fig. 2). The migration time is long with the anodal EOF and peak efficiency is lower (Fig. 2b) than in the run with cathodal EOF (Fig. 2a). The presence of the surfactant as buffer additive may cause problems when mass-spectrometrie is used, or a collection of microfractions of resolved zones is required. Additional problem with the dynamic coating is a poor reproducibility of the migration times. The use of a capillary with permanent inner wall coating may allow to avoid some of these problems.

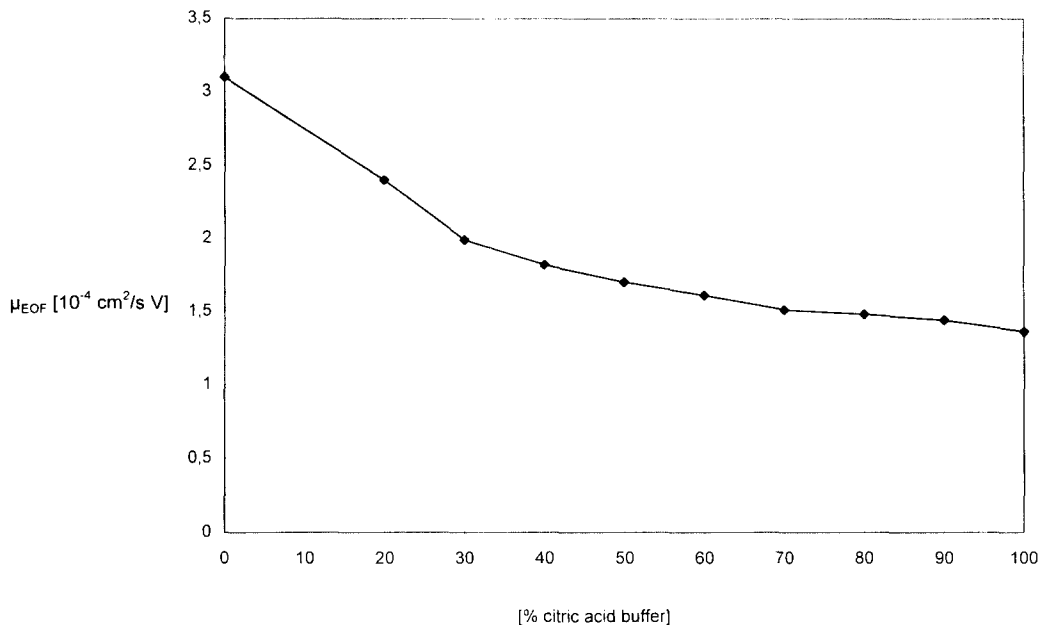The use of the PEI-coated capillary [23] for the reversal of the EMO of thalidomide is shown in

Fig. 4. Dependence of the EOF on the composition of a citric acid/succinic acid buffer at pH 3.0. Conditions: 50 mM citric acid and succinic acid buffer. Capillary with a positively charged inner surface (50 μm) with 60 cm total length and 43 cm effective length.

Fig. 3. The migration times in the 'reversed' mode (Fig. 3b) are well reproducible and comparable with those in the 'direct' mode (Fig. 3a) in this capillary. Peak efficiency is high and an interaction between the chiral selector and surfactant in a bulk solution is precluded. However, the interaction of the chiral selector with the surfactant which is immobilized on the capillary wall cannot avoid in this technique. A disadvantage of this mode is that two different capillaries are required for a 'direct' and a 'reversed' run. The use of capillaries with the pH dependent switchable anodal/cathodal EOF [29–31] seems very useful in order to avoid the last disadvantage of a permanently coated capillary.

This is a general technique for the reversal of EMO of neutral chiral analytes in CE and in this study this was confirmed also with benzoin and 5-methyl-5-phenyl-hydantoin as chiral analytes.

4.2. Reversal of EMO of a cationic analyte

Recently, a technique was described for the reversal of the EMO in uncoated fused-silica cap-

illaries [4]. This technique is based on a reverse of the relation between the oppositely directed EOF and self-electrophoretic mobility of analytes and requires that the charge on the capillary inner wall and the charge of the analyte have the same sign. Therefore, in an uncoated silica capillary with a negative surface-charge this technique is feasible only for anionic chiral analytes. However, this technique can cover cationic analytes too in the capillaries with a positively charged inner surface.

The PEI-coated capillary with a permanent anodal EOF was used in this study in order to observe a pH dependent reversal of the EMO for a cationic analyte dimethindene. The analyte should be injected on the anodic end of the separation capillary and detected on the cathodic side of that, when $\mu_{EOF} < \mu_{el}$, and it should be injected on the cathodic end of the capillary and detected on the anodic side of the separation capillary when $\mu_{EOF} > \mu_{el}$.

The general problem is that the anodal EOF in a PEI-coated capillary and the electrophoretic mobility of a cationic analyte both tend to change by similar way with pH. For instance, both of

them decrease with increasing pH. On the other hand, the practical realization of this technique requires an existence of a cross point on the pH dependence curves of μ_{EOF} and μ_{el}. This means that the anodal EOF should be significantly lower than μ_{el}, at low pH, to allow the detection of a sample on the cathodic side of the separation capillary in a reasonable time and it should be significantly higher than μ_{el} at higher pH to allow the detection of the cationic analyte on the anodic side of the separation capillary. Coated capillaries containing quaternary ammonium ions can be useful to manage a lower degree pH-dependent or pH-independent anodal EOF.

A dependence of anodal EOF in a PEI-coated capillary on the composition of a citric acid/succinic acid buffer at pH 3.0 is depicted in Fig. 4. As shown in this figure, a desirable anodal EOF can be easily adjusted by changing the composition of a buffer.

The reversal of the EMO of the cationic analyte dimethindene is shown in Fig. 5. This technique can also be used for other cationic analytes providing that the pHs and the buffer compositions are appropriately chosen.

Acknowledgements

Fig. 5. Reversal of the migration order of [S-(+): R-(−) = 3:1] mixture of dimethindene in capillary with positively charged inner surface at pH 3.0 (a) and pH 5.3 (b). Capillary (50 μm) with 60 cm total and 43 cm effective length was used. Field strength 400 V cm^{-1}, 50 mM phosphate buffer, 5 mg ml^{-1} β-CD and injection at the anode in case (a). Field strengh 500 V cm^{-1}, 50 mM acetate buffer, 3 mg ml^{-1} β-CD and injection at the cathode in case (b).

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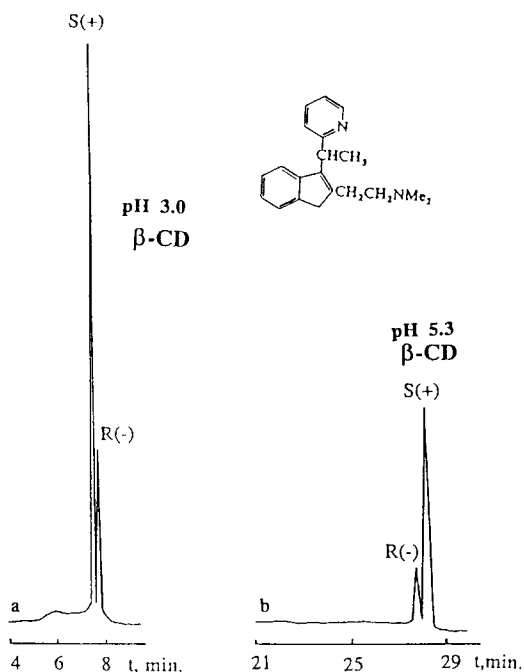[17] N.D. Stepanova and A.V. Stepanov, Zhurnal Prikladnoi Khimii, Russian J. Appl. Chem., (English edn.), 42 (1969) 1576–1578.

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