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Separation of basic solutes by reversed-phase capillary electrochromatography

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Abstract

The separation of basic solutes at low pH by capillary electrochromatography (CEC) has been investigated. The feasibility of separation of basic solutes by CEC was demonstrated. Influence of operational parameters, solvent composition, pH, temperature on retention and selectivity of the separation of a mixture of basic, neutral and acidic drug standards has been investigated. The observed elution behavior has been modeled to account for both chromatographic retention and differential electrophoretic mobility of the solutes. This model was verified experimentally. It is demonstrated in this work that the elution window of solutes in reversed-phase CEC is expanded to range from -1 to ∞. © 2000 Published by Elsevier Science B.V.

Keywords: Electrochromatography; Retention models; Basic solutes

1. Introduction

In the field of separation science, capillary electrochromatography (CEC) is at this moment one of the most exciting new developments. Since in the early 1990s, the feasibility of this methodology was established unambiguously [1–12], many groups have started to work on specific aspects of CEC with the intention to improve the methodology or work around limitations.

Separation of basic solutes by reversed-phase (RP) CEC has been perceived as one of the limitations of the technique [13]. This is understandable for the following reasons. In CEC, like in high-performance liquid chromatography (HPLC), the solvent needs to be propelled through a packed bed or open-tube in order to allow the dissolved solutes to partition and retain on the stationary phase. Therefore the presence of an electroosmotic flow (EOF) is a mandatory requirement for CEC. This EOF is generated in a capillary packed with silica-based reversed-phase particles or in a (coated) fused-silica capillary through the presence of an electrical double layer at the solid–liquid interface in case the capillary is filled with an electrolyte. In the case of silica surfaces the basis for the built-up of an electrical double layer, are ionizable silanol groups at the surface of the particle–capillary which provide a negative charge, depending on the pH of the electrolyte. In the early days of silica-based, reversed-phase type HPLC though, it has been found that the separation of strongly basic solutes (containing aliphatic or alicyclic amine groups) leads to high retention, bad peak shape and – sometimes – complete adsorption of these solutes [14]. Köhler and Kirkland demonstrated that this behavior had to be attributed to unreacted silanol groups at the surface of these, first-generation RP stationary phases [15].

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The silanol groups have widely differing acidity depending on their chemical structure (isolated, geminal or vicinal silanol groups) and strong ionic (coulombic) and/or polar (hydrogen bonding) interactions with amine solutes depending on the pH of the mobile phase and the pK_a of the solute. Thus the same silanol groups that are required to generate an EOF in CEC and which are present on the surface of first generation RP stationary phases though are expected to be detrimental for elution and separation of basic solutes in CEC.

Köhler and Kirkland's work has led to the emergence of a wide palette of second-generation silica-based stationary phases with weakly acidic silanols and which are well shielded by the type of bonding or by exhaustive end-capping.

It was recognized early in the development of HPLC, that the reduction of the pH of the mobile phase (2.5–4.5) was one way to mitigate unwanted interactions. Silanol groups will be neutralized at a low pH and electrostatic interactions with the basic solutes will be suppressed. But in case one reduces the pH of the mobile phase in CEC, the EOF generated will be reduced because the silanol groups generating the surface charge are neutralized. So reduction of the pH may not be an option for separation of basic solutes by CEC.

On the other hand, reviewing the work done with CEC so far, the majority of separations have been executed on so-called first-generation silica-based reversed-phase stationary phases. Besides a substantial number of unreacted silanol groups, the base silica matrices have a relative high concentration of cationic (Na^+, K^+, Ca^{2+}, Al^{3+} and Fe^{3+}) and anionic (SO_4^{2-}, carbonate, borate and aluminate) species [16,17]. Such ionic contaminants may well contribute to the surface charge and therefore to the EOF of such phases. In addition these contaminants may have an adverse effect on retention and peak shape of basic solutes as was demonstrated in HPLC unambiguously [16,17]. So it was not surprisingly that our first attempt to ''separate'' a basic solute, imipramine, under conditions regarded to be optimal for CEC, viz. pH 8 on a first-generation silica-based reversed-phase stationary phase, Spherisorb ODS-I rendered an unsatisfactory result (Fig. 1). Under the conditions given in Fig. 1, typically an EOF of approx. 2 mm/s is obtained. As can be observed the imipramine is retained but shows poor peak shape. If one repeats this experiment with the same stationary phase packed in an HPLC column at the same linear velocity, an almost identical result (plate number, peak shape and retention) is obtained.

This result is typical for the separation of strongly basic solutes by CEC under these conditions. Less strongly basic solutes, e.g., triazines show better

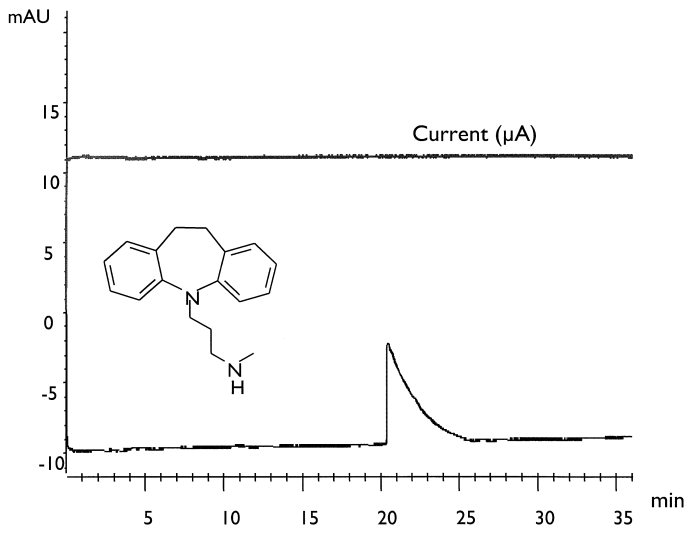

Fig. 1. Elution of imipramine under CEC conditions. Column, 250(335)×0.1 mm, Spherisorb ODS-I, 3 μm. Mobile phase, ACN–50 m*M* Tris–HCl, pH 8 (4:1). Temperature: 20°C. Voltage: 20 kV. Detection: 250:100 nm.

peak shape and high efficiency [18]. But for strongly basic solutes this approach seems not very promising.

Other workers have applied a cation-exchange type stationary phase for the separation of basic solutes at pH clearly below the pK_a of these solutes [19,20]. Their results have generated some controversy because impressive peak compression leading to high efficiencies of the bands was obtained besides adequate retention. These compression effects are of electrophoretic origin, have not been very reproducible so far and not yet well described in a comprehensive manner. Therefore this approach needs further work.

Usage of a polar stationary phase with polar solvents has been applied for the separation of basic solutes in HPLC. Therefore some groups have tried to transfer such methods to CEC with bare silica as a stationary phase [21,22]. This work has also not yet led to a convincing solution for the separation of basic solutes by CEC and needs more attention and research.

The addition of a competing amine to the mobile phase at low pH, which scavenges the ''hot'' silanol groups on RP stationary phases, has been proposed to improve the separation of basic substances by HPLC [23–26]. Lurie et al. were the first to recognize the potential of this approach for the separation of basic solutes by RP-CEC [27]. Working at low pH with hexylamine as the competing amine added to the mobile phase they showed improved significantly improved peak shape for basic solutes while maintained a low EOF. Others soon confirmed their results [28,29]. This approach looks very promising in many aspects. Good peak shape, and high efficiency for basic solutes is obtained. But particularly attractive is Lurie et al.'s method because two separation mechanisms (chromatographic retention and electromigration) are combined onto one separation column, which provides a unique ability to tune the selectivity of separation to requirements.

In this paper the authors describe their work in systematic evaluation of operational parameters of the Lurie et al. method for the separation of basic solutes by RP-CEC viz. concentration of the amine modifier, concentration of the organic solvent, temperature and type of stationary phase. Based on these results, a model has been developed which combines the contributions to separation by electromigration and partitioning of the solutes in a manner that allows prediction of elution times in case properties change. Experiments that confirm the validity of the model have been executed.

2. Experimental

2.1. Chemicals

The aqueous buffers used were (tris-hydroxymethyl)aminomethane (Tris), 2-(*N*-morpholino)-ethanesulfonic acid (MES), sodium acetate (NaOAc) and phosphoric acid (H_3PO_4) all from E. Merck (Darmstadt, Germany). *n*-Hexylamine was also obtained from E. Merck. The buffers were adjusted to the desired pH using either HCl or NaOH (E. Merck). The buffer according to Lurie et al. [27] was prepared by adding the appropriate volume of *n*-hexylamine to a 25 m*M* diphosphate buffer and adjusting the pH to the desired value with phosphoric acid.

Acetonitrile (ACN) (J.T. Baker, Deventer, The Netherlands) was used as organic modifier. The eluents were prepared by first adjusting the buffer to the desired pH, then mixing with the appropriate amount of organic modifier.

The sample compounds were thiourea, dimethyl phthalate, diethylphthalate, biphenyl and *o*-terphenyl (all from E. Merck) for the neutral test mixture. Imipramine, procaine, ambroxol, metaclopramine, timolol, antipyrine and naproxen (all from Sigma–Aldrich–Fluka, Deisenhofen, Germany) were used in the pharmaceutical standard test mixture. The structures of these solutes are given in Fig. 2. Samples were prepared by mixing the appropriate buffer with a stock solution of approx. 20 mg/compound in 100 ml acetonitrile to the same acetonitrile:buffer ratio as the respective eluent.

2.2. Columns

All capillaries were packed according to a slurry packing procedure described in detail before [3]. Polyimide coated fused-silica tubing obtained from Polymicro Technologies (Phoenix, AZ, USA) was of 100 μm I.D.×360 μm O.D. A packed bed length of

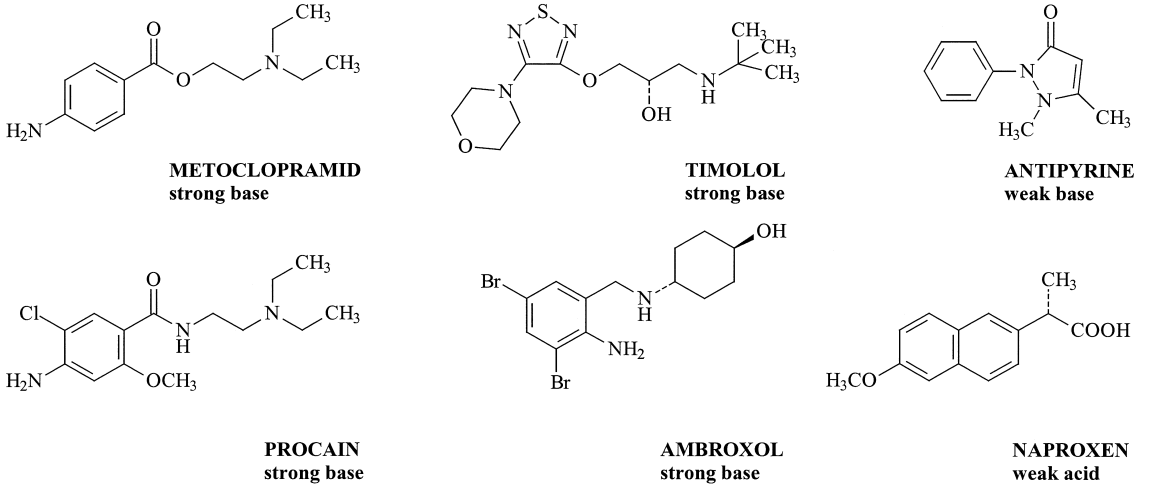

Fig. 2. Structures of the components of the drug standard test mixture.

25 cm was prepared, total column length was 8.5 cm plus packed bed length [indicated in all the figures and text as 250(335) mm]. Packing materials were obtained from Hypersil (Runcorn, UK) (CEC Hypersil-C_{18}, 3 μm and CEC Hypersil-C_8, 3 μm) and Waters Phase Separations (Clywd, UK) (Spherisorb ODS-I, 3 μm).

2.3. Instrumentation

Unless otherwise mentioned, CEC chromatograms were obtained with the Hewlett-Packard HP^{3D}CE (Hewlett-Packard, Waldbronn, Germany) instrument with the option to apply a pressure of 10–12 bar to the outlet and/or inlet vial. Throughout the work the pressurization option of the instrument was used to prevent formation of gas bubbles in the capillaries. After packing, columns were directly put into the HP^{3D}CE instrument and flushed with the run buffer electroosmotically for ca. 30 min before the first run. Changing eluents was also done electroosmotically. In the rare case that parts of a column had dried out, this column was purged on a HP 1050 pump (Hewlett-Packard) for ca. 30 min at a pressure of ca. 80 bar to remove all air bubbles from the column. Samples were injected electrokinetically (5 kV for 3 s). Detection wavelength was 250 nm with a bandwidth of 100 nm unless specified differently in the figure legend. Solute identity was confirmed by UV–Vis spectra obtained with the diode-array detector. High voltage was applied as a 3–6 s time ramp to avoid stress to the column. The CEC chromatograms demonstrating the LC–CEC mixed mode operation were obtained on a Hewlett-Packard prototype HP^{3D}CE instrument equipped with a modified cassette connected to a HP 1100 binary pump [30]. In this prototype system a flow is delivered to the inlet vial of the HP^{3D}CE system hydraulically where at the same time a voltage can be applied.

3. Results

The magnitude of the EOF is the first concern, when applying a low pH solvent in CEC as explained in the Introduction. Therefore, in a first experiment, a mixture of neutral test solutes was applied while using the *n*-hexylamine-containing, low-pH buffer eluent (Fig. 3).

In Fig. 3, the lower trace shows the chromatogram obtained at pH 8. It represents a quasi-standard result for CEC with efficiency of all peaks around 200 000 plates/m and an EOF 1.6 mm/s. The upper trace shows the chromatogram obtained with the so-called Lurie buffer. Now the EOF has decreased significantly to 0.5 mm/s. The efficiency of all peaks is still excellent and in line with expected values at the lower mobile phase velocity. The existence of an

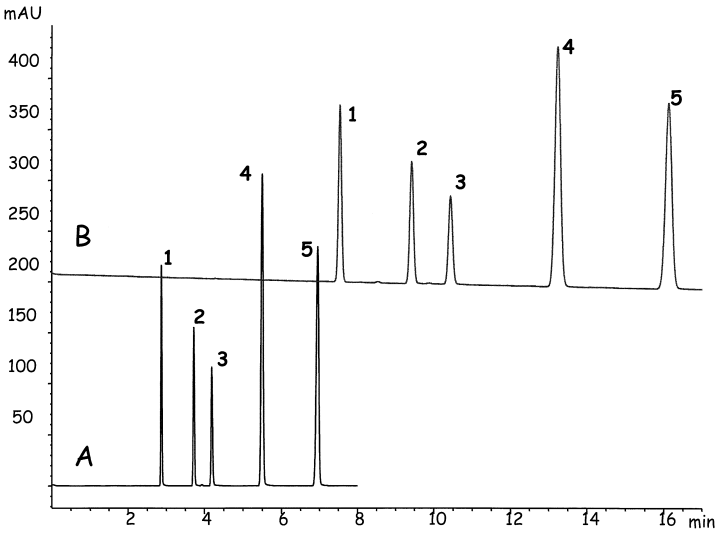

Fig. 3. CEC separation of a neutral test mixture at pH 8 and pH 2.5. Column, Waters Spherisorb ODS I, 3 μm, 250(335)×0.1 mm, (A) acetonitrile–25 m*M* Tris, pH 8 (4:1), (B) acetonitrile–25 m*M* phosphate, 0.2% hexylamine, pH 2.5 (4:1); voltage: 25 kV, temperature: 20°C. Peaks: 1=thiourea, 2=dimethylphthalate, 3=diethylphthalate, 4=biphenyl, 5=*o*-terphenyl.

EOF under these conditions is surprising and needs clarification. At low pH it was argued before that all silanols are neutralized and do not contribute to surface charge. Therefore, the existence of EOF indicates that there are other contributions to surface charge on this type of silica than just silanols. The surface charge may be attributed to the presence of inorganic, anionic species in the silica. This hypothesis is supported by our observations that second-generation silica-based reversed-phases, which have much higher purity, do not show EOF at low pH values [30].

The low EOF under these conditions though still is high enough for chromatographic work. Therefore in a next experiment, the drug standard test mixture described in Fig. 2 was applied to the Spherisorb ODS-I and two other CEC columns. The result is given in Fig. 4.

The three traces reveal a similar pattern. Under the selected conditions, all strongly basic solutes, procaine (1), timolol (2), ambroxol (3) and metoclopramide (4) elute before the dead time marker, thiourea (5). The weakly basic antipyrine (7) and the neutralized weakly acidic naproxen (6) are retained and elute after t_0. Comparison of the chromatograms obtained on each column reveals best efficiency and peak shape on the Spherisorb ODS-I column and partly tailing peaks for 1–4 on the Hypersil column. On the CEC Hypersil-C_8 column, antipyrine did not elute.

The velocity of the strong basic solutes is obviously higher than the EOF. Chromatographic retention of these cationic species is low under these conditions (80% acetonitrile, see also Fig. 5). So the combined action of EOF and electrophoretic mobility moves the cationic solutes ahead of the dead time marker. The selectivity of separation though is improved by stationary phase interaction as will be shown in the next paragraph. Antipyrine is partly ionized under these conditions. Therefore its acceleration by the electrical field is much lower and retention by partitioning higher than the strongly basic substances and antipyrine elutes as the last solute. Thus effectively, a group type separation is obtained, with the strongly basic solutes eluting before, and the acidic and neutral solutes eluting after t_0.

In order to illustrate the effect of chromatographic retention on the selectivity of the separation under electro-drive conditions in a next experiment, the drug sample was separated on the Spherisorb ODS-I column under pressure-drive conditions (Fig. 5). In

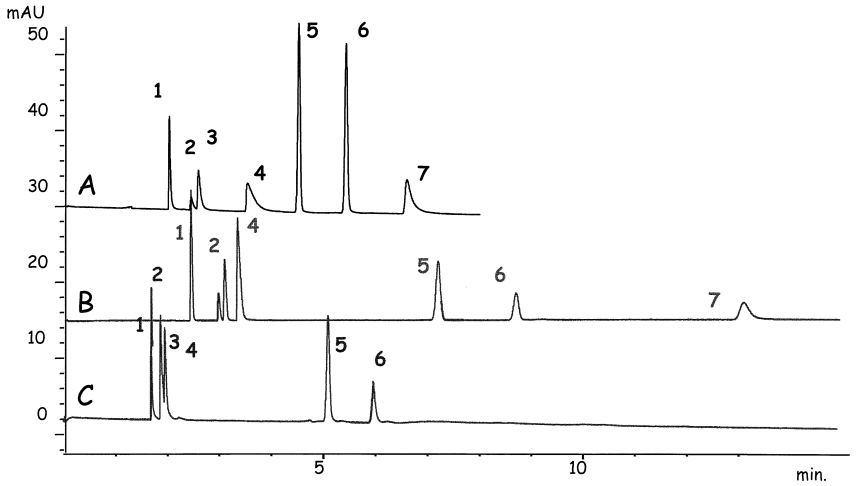

Fig. 4. CEC separation of a drug standard test mixture with Lurie's buffer. Columns, 3 μm, 250(335)×0.1 mm, (A) Spherisorb ODS-I, 3 μm, (B) CEC Hypersil-C_{18}, (C) CEC Hypersil-C_8; eluent: ACN–25 m*M* phosphate, 0.2% hexylamine, pH 2.5 (80:20), voltage: 25 kV, temperature: 20°C. Peaks: 1=procaine, 2=timolol, 3=ambroxol, 4=metoclopramide, 5=thiourea, 6=naproxene, 7=antipyrine.

contrast, a pure CZE separation of the sample was done with the solvent used in the CEC and LC experiments. The result is shown in Fig. 5.

For the μHPLC experiment, a modified $HP^{3D}CE$ system was used, which allowed the introduction of the mobile phase to the inlet of the packed capillary by an HP 1100 series HPLC binary pump [30]. In this experiment, middle trace, the dead time marker thiourea is the first peak that elutes (velocity 1 mm/s). The neutral solutes, naproxen (6) and antipyrine (7) elute at similar relative retention as in the CEC experiment (upper trace). k' is 0.2 for naproxen

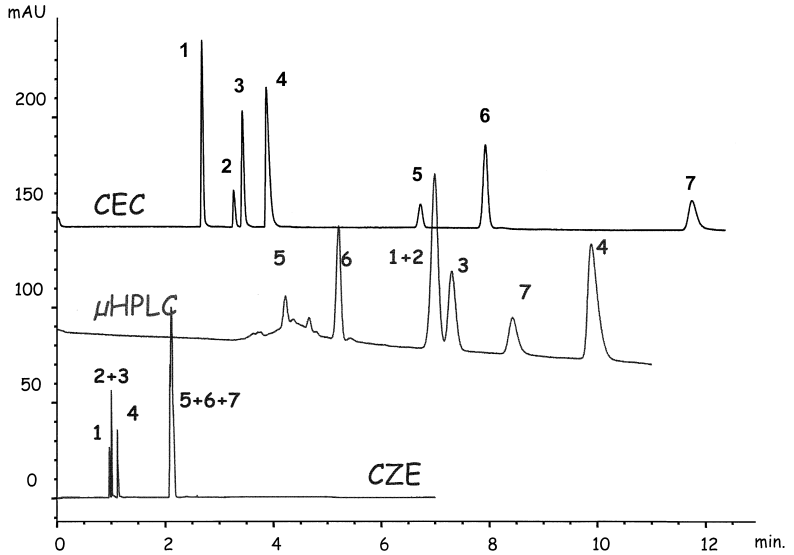

Fig. 5. Comparison of separation of drug mix by CEC, HPLC and CZE. Column, Spherisorb ODS-I, 3 μm, 250(335)×0.1 mm. Solvent as in Fig. 4. Voltage: 25 kV, pressure (HPLC), 200 bar. CZE, uncoated fused-silica capillary 250(335)×0.075 mm.

in both experiments and 0.8 and 0.9 for antipyrine in the CEC and HPLC experiment, respectively. But in the LC experiment, all strong basic solutes elute after t_0. In the CZE experiment, the strong basic solutes all move ahead of all neutral ones which co-elute. But in comparing the upper trace with the lower trace in Fig. 5, one can clearly establish, that chromatographic interactions have caused better separation of the strong basic solutes than in the capillary zone electrophoresis (CZE) mode. This finding substantiates the hypothesis that the separation of the drug standards obtained in the CEC mode, results from both electrophoretic and partitioning separation mechanisms.

With this initial result established, the influence of variation of operation parameters on retention and selectivity of the separation was investigated in a more systematic manner. In Fig. 6, the influence of change in the percentage of organic modifier and pH of the aqueous buffer is illustrated.

In the left panel of Fig. 6, the percentage acetonitrile in the mobile phase was reduced from 80% to 60%. As expected, retention increases for solutes 6 and 7. Quite remarkable though, the increase in retention for antipyrine is less than the retention increase for naproxen. Tentatively this is interpreted being caused by the small residual electrophoretic velocity of antipyrine, which despite its low pK_a value will have a fractional, positive charge. By the same reasoning, the same shift happens at a pH 3.8 of the aqueous buffer. The retention and selectivity of the strong basic solutes (1, 2, 3 and 4) did not change much on changing the organic modifier concentration however.

With reduction of the percentage of acetonitrile in the mobile phase, the EOF reduces. This is in line with other reports [18], i.e., EOF decrease with increase of the percentage of aqueous buffer in the mobile phase in CEC. At pH 3.8, the EOF is slightly higher than with the corresponding composition but at pH 2.5. This is also expected.

Temperature has a less dramatic effect on retention and selectivity of separation for this sample (Fig. 7).

As expected, retention decreases at the higher temperature but there are no changes in the selectivity of the separation.

Next the influence of the concentration of the amine modifier on retention and selectivity of separation of the drug mix was investigated. This was done with a CEC Hypersil-C_{18} column. On this column some of the basic solutes have shown poor peak shape under the conditions used initially. Therefore both the pH and the concentration of hexylamine in the aqueous buffers were varied simultaneously. The result is given in Fig. 8.

It can be seen that the increase of the concentration of hexylamine in the aqueous buffer, did improve the peak shape of the basic solutes. At the same time, a large change in retention of the strong

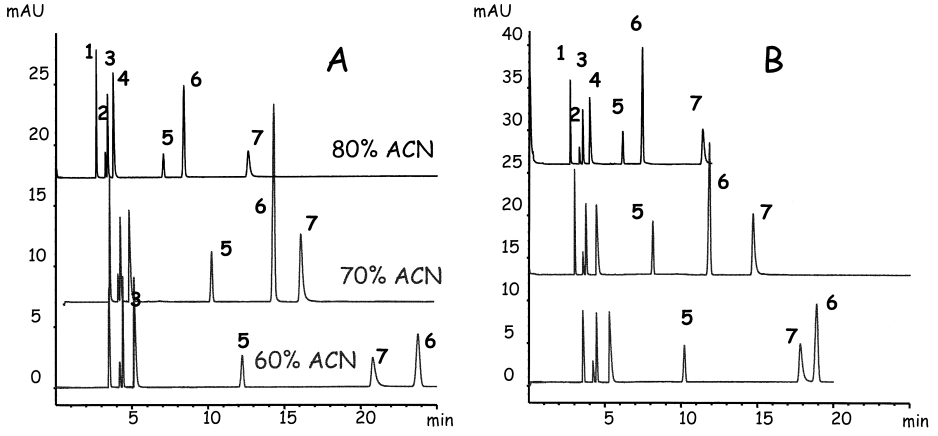

Fig. 6. Influence of percentage organic modifier and pH of the aqueous buffer on retention and selectivity. Column, Spherisorb ODS-I, 3 μm, 250(335)×0.1 mm; mobile phase as in Fig. 4, (A) pH 2.5, (B) pH 3.8. Peak identification as in Fig. 4.

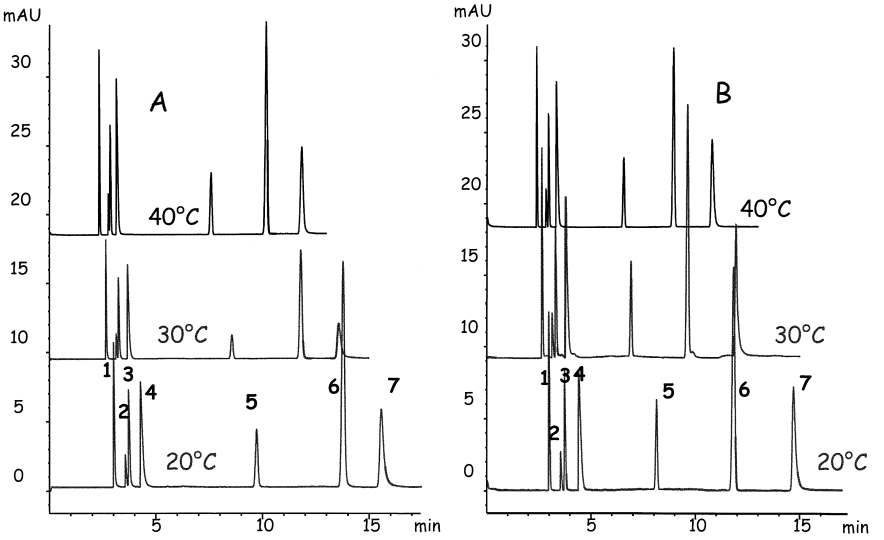

Fig. 7. Influence of temperature on retention and selectivity of separation of the drug standard sample. Column, Spherisorb ODS-I, 3 μm, 250(335)×0.1 mm; eluent, acetonitrile–25 m*M* phosphate 0.2% hexylamine, pH 2.5 (7:3); (A) pH 2.5, (B) pH 3.8; voltage: 25 kV; temperature: see figure; peak identification as in Fig. 4.

basic solutes occurred (compare the two lower traces in Fig. 8). In addition the EOF decreased quite significantly. One may conclude from this observation that the type and concentration of the amine modifier in these cases will have to be selected carefully and may differ substantially by stationary phase type.

In a final experiment, the μHPLC separation of the drug mix was compared with a field assisted μHPLC separation. For that purpose the HP3DCE

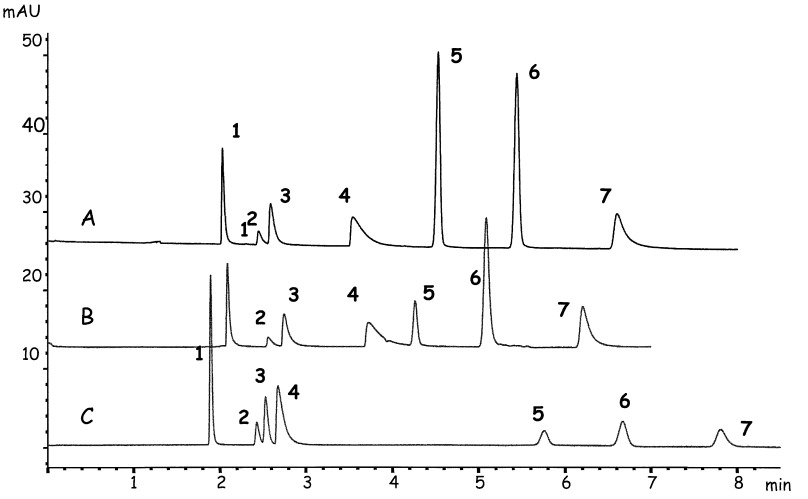

Fig. 8. Influence of pH and amine modifier concentration on retention and selectivity of separation of the drug standard sample. Column, CEC Hypersil-C_{18}, 250(335)×0.1 mm, 3 μm; eluent, acetonitrile–25 m*M* phosphate (8:2), (A) 0.2% hexylamine, pH 2.5, (B) 0.2% hexylamine, pH 3.8, (C) 1.0% hexylamine, pH 3.8; voltage: 25 kV; temperature: 20°C; peak identification as in Fig. 4.

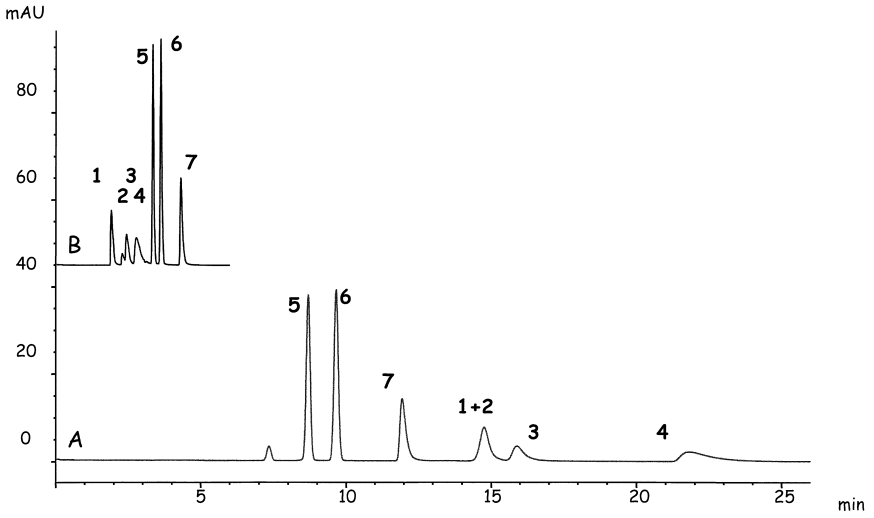

Fig. 9. μHPLC versus ''E-ssisted'' μHPLC separation of drug standard. Column, CEC Hypersil-C_{18}, 3 μm, 250(335)×0.1 mm; eluent, ACN–25 m*M* phosphate, 0.2% hexylamine, pH 2.5 (80:20); voltage: 25 kV; temperature: 20°C; peak identification as in Fig. 4. (A) Pressure 210 bar, voltage 0 kV, (B) pressure 210 bar, voltage 25 kV.

prototype system described earlier was used [31]. With this system one can deliver an hydraulic flow to the column inlet at a maximum pressure of about 300 bar and at the same time applied a voltage (''E-ssisted'' HPLC). So now, hydraulic and EOF flow will cooperate to accelerate the separation. Moreover it can be expected still that the strong basic solutes will be faster than the velocity of the solvent and therefore elute before t_0. This is exactly was happens as can be seen in Fig. 9.

4. Retention model of separation by combined electromigration and partitioning

The previous results have revealed that in CEC of charged, in this case cationic, solutes retention and selectivity of separation are governed by the electrophoretic mobility of the solutes and partitioning between mobile and stationary phase as in RP-HPLC. Retention in HPLC is described by the simple relationship given in Eq. (1):

$$t_R = t_0(1 + k') \tag{1}$$

In Eq. (1), t_R is the measured retention time of the solute, t_0 is the time a non-retained solute needs to transverse the column and k' is the capacity factor. In CEC, however, as has been demonstrated in this work, elution of compounds is determined by partitioning and electrophoresis, thus Eq. (1) needs to be modified to [32]:

$$t_{el} = (1 + k'_{LC}) \cdot t_0 \cdot \frac{t_{ep}}{t_{ep} + t_0} \tag{2}$$

Here t_{el} is the elution time of a specific solute, t_0 is the elution time of an unretained and uncharged solute, k'_{LC} is the k' value associated with mobile phase partitioning and t_{ep} the elution time of the solute in pure electrophoresis mode.

The first part of Eq. (2) describes the chromatographic retention. The second part describes the modulation of solute velocity due to electromigration. In the case of an uncharged solute t_{ep} will equal infinity and Eq. (2) is reduced to Eq. (1). In case, however, the solute has a significant mobility, the factor will become smaller than 1 and the measured elution time is reduced. For example, if the solute has a mobility equal to the mobile phase, the factor equals 0.5 and the overall elution time in CEC mode will be halved compared to pure HPLC.

Similar to the capacity factor in HPLC we can define a capacity factor for CEC:

$$k'_{CEC} = \frac{t_{el} - t_0}{t_0} \quad (3)$$

Although the definition of k'_{CEC} is very similar to k'_{LC} it has to be kept in mind that the physical meaning of these two capacity factors is quite different. In liquid chromatography the capacity factor describes retention of a solute due to partitioning and is directly proportional to the distribution constant of the solute in stationary and mobile phase. The k'_{CEC} describes the elution of a solute relative to an uncharged and unretained solute and depends on both, partitioning and electromigration. While the k'_{LC} can assume values between 0 and infinity, the k'_{CEC} can also assume negative values (in case a solute elutes before t_0).

After substitution of Eq. (2) in Eq. (3) and proper rearrangement the following equation is obtained.

$$k'_{CEC} = \left(1 - \frac{u_{ep}}{u_0 + u_{ep}}\right) \cdot k'_{LC} - \frac{u_{ep}}{u_0 + u_{ep}} \quad (4)$$

In Eq. (4), u_{ep} is the electrophoretic velocity of the solute and u_0 is the mobile phase velocity (which actually may be pressure or electrical driven). The term $u_{ep}/(u_0 + u_{ep})$ describes the relative migration in CE and is identical to the reduced mobility μ_r defined by Schwer and Kenndler [33]:

$$\mu_r = \frac{u_{ep}}{u_{ep} + u_0} \quad (5)$$

Substitution of Eq. 5 in Eq. (4) provides,

$$k'_{CEC} = k'_{LC} - \mu_r \cdot k'_{LC} - \mu_r \quad (6)$$

which describes how the chromatographic k' is modulated by the electrophoretic mobility of the solute. In the limiting case that the solute does not have an electrophoretic mobility, k'_{CEC} equals k'_{LC} as expected. Alternatively, in case a solute does not have chromatographic retention and k'_{CEC} equals the reduced mobility (Eq. (7)):

$$u_{ep} = 0 \quad k'_{CEC} = k'_{LC}$$

$$k'_{LC} = 0 \quad k'_{CEC} = \frac{u_0}{(u_0 + u_{ep})} - 1$$

$$= \frac{u_{ep}}{(u_0 + u_{ep})} = -\mu_r \quad (7)$$

In the limiting case that t_{el} approaches 0 (high electrophoretic mobility in the same direction as the mobile phase, no chromatographic retention), the minimal value for k'_{CEC} approaches -1. Thus the retention window for a separation in CEC is given in Eq. (8).

$$-1 < k'_{CEC} < \infty \quad (8)$$

Using Eq. (6), one can start to model the effects of chromatographic retention, electrophoretic mobility and the velocity of the mobile phase (driven electrically or hydraulically) to optimize a separation.

In a practical case, one will have to do two experiments in order to predict retention of a charged solute in CEC viz. an HPLC separation to establish k'_{LC} and a CZE experiment to find μ_{ep} which is the electrophoretic mobility of the solute from which μ_r in the CEC mode can be calculated. The electrophoretic mobility of the solute is a physical constant and independent of the geometry of volume in which it is measured. Obviously in both cases the same solvent must be used.

The model was verified by prediction of the elution times of the standards in a CEC run from the k'_{LC} and k'_{CE} obtained from μHPLC and CZE separation of the solutes. The CZE experiment was done with a polyvinylalcohol (PVA)-coated capillary in order to minimize secondary interactions of the basic solutes with the capillary wall, which would lead to false determination of their mobilities. The run buffer used was identical with the mobile phase in the CEC and HPLC experiments.

As can been seen from Table 1, an excellent match between expected and experimental values of the k'_{CEC} was found. This validates the model well.

In a next step the approach was taken one step further. The model description given in Eq. (6) was used to visualize how the selectivity of separation is affected when the electrophoretic mobility and capacity factor changes.

The simulation was done with MS EXCEL. By entering appropriate values for mobility and the capacity factor of the solutes, their elution time is calculated. The bands are supposed to have an arbitrary height and are convoluted with a simple Gaussian distribution to obtain a chromatogram.

Six sample components were used. The electro-

Table 1
Comparison of calculated with experimental values of k'_{CEC} for three different CEC columns[a]

Solute	k'_{LC}	μ_r	k'_{CEC} calculated	k'_{CEC} found
CEC Hypersil-C_{18}				
Procaine	0.70	0.74	−0.56	−0.57
Timolol	0.70	0.68	−0.46	−0.48
Ambroxol	0.83	0.68	−0.41	−0.44
Metoclopramide	1.51	0.73	−0.32	−0.32
Naproxen	0.11	0.03	0.08	0.09
Antipyrine	0.37	0.06	0.29	0.30
CEC Hypersil-C_8				
Procaine	0.37	0.75	−0.66	−0.66
Timolol	0.32	0.69	−0.59	−0.61
Ambroxol	0.32	0.69	−0.59	−0.61
Metoclopramide	0.46	0.74	−0.62	−0.63
Naproxen	0.22	0.03	0.18	0.17
Antipyrine	0.32	0.06	0.24	n.a.
Spherisorb ODS-I				
Procaine	0.61	0.81	−0.69	−0.66
Timolol	0.61	0.76	−0.61	−0.59
Ambroxol	0.67	0.76	−0.60	−0.57
Metoclopramide	1.24	0.80	−0.55	−0.54
Naproxen	0.25	0.04	0.2	0.21
Antipyrine	1.00	0.08	0.84	0.82

[a] Experimental conditions as in Fig. 4.

phoretic velocities and capacity factors of the components are given in Table 2. Fig. 10a shows a simulated separation of these six components in CEC mode with only EOF, Fig. 10b shows the same separation with a hydraulic flow of −1 mm/s added. In the simulation we have three solute pairs having the same mobility and two groups solutes having the same k'_{LC}. Thus in a CE separation we would get three peaks while in an HPLC separation we would get two peaks. Due to the unique separation properties of CEC we obtain separation of all six components in CEC mode. Based on their high electrophoretic mobility components 1–4 elute before the t_0 marker although components 3 and 4 are retained. It is thus possible to perform group type separations that elute, e.g., strongly basic components well ahead of the neutral species.

Table 2
Electrophoretic mobilities and k'_{LC} values for simulated electrochromatogram

	u_{ep}	k'_{LC}	t_{el} (s)	t_{el} (s)
Peak 1	3.3	0	43.1	52.1
Peak 2	3.0	0	45.5	55.7
Peak 3	3.3	0.5	64.7	78.1
Peak 4	3.0	0.5	68.2	83.3
Peak 5	0	0	100	166
Peak 6	0	0.5	150	250

Fig. 10b demonstrates the possibilities of the addition of pressure driven flow in CEC. As can be seen from the definition of the reduced mobility μ_r the magnitude of this term can be changed if the electrophoretic mobility u_{ep} and the solvent mobility u_0 can be changed independently. This cannot be achieved in a purely electro-driven system. With the addition of hydrodynamic flow, however, only u_0 is changed while u_{ep} remains the same. By adding a hydrodynamic counter flow of 1 mm/s, the resolution of solvent pairs 1/2 and 3/4 is increased from 2.7 in the pure electro-driven case to 3.2 in the mixed flow mode.

This model can be used easily for optimization of separation once the mobility of the particular solutes

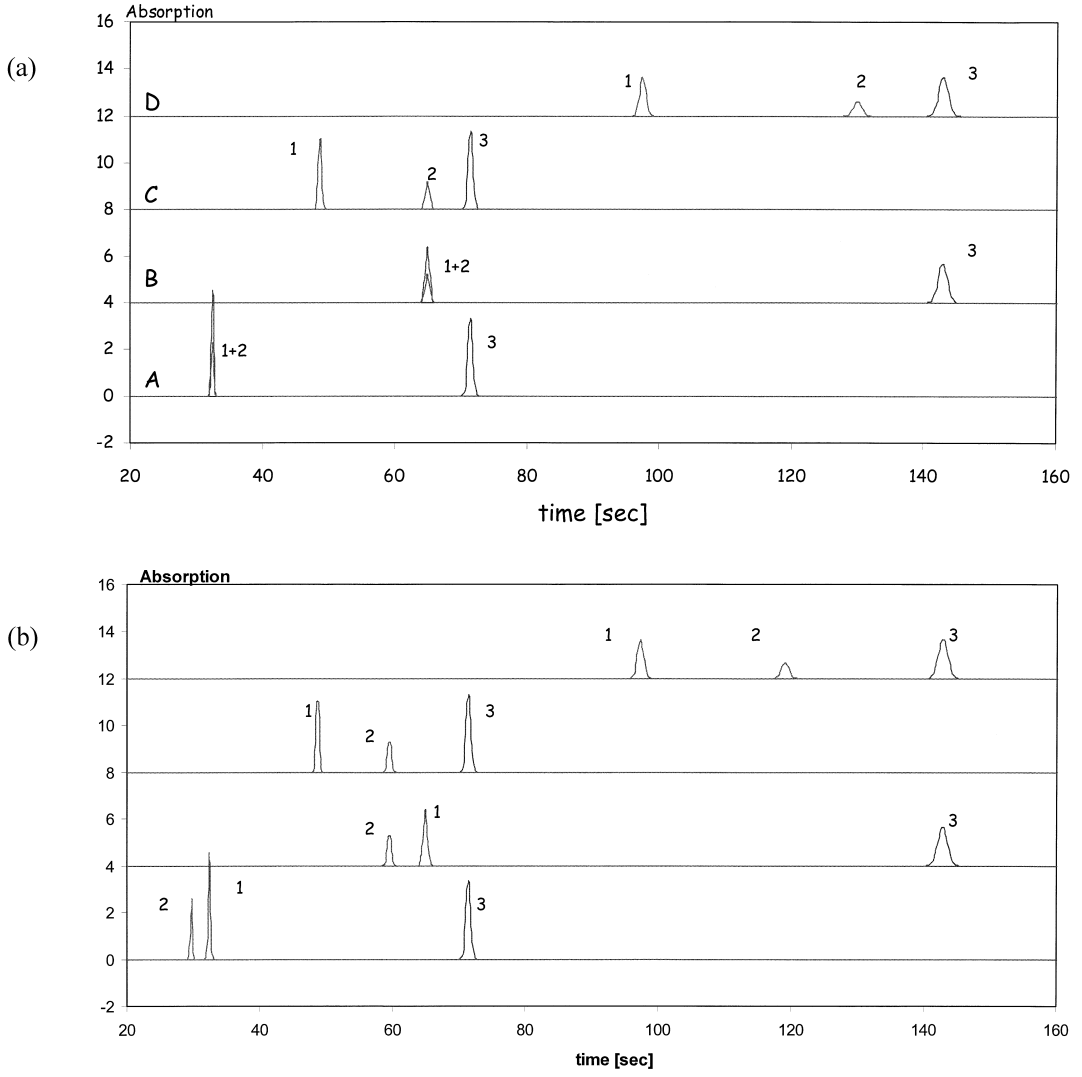

Fig. 10. Modeling separation of solutes by electromigration and retention. Conditions: mobility of solvent 0.00025 $cm^2/V\,s$, field 1000 V/cm, column 250(335) mm, plate number 40 000, (a) EOF only (2.5 mm/s), (b) EOF (2.5 mm/s) with additional hydraulic flow (−1 mm/s).

is known. The k' can be adapted easily but changing the organic solvent content. In a next publication, this will be verified extensively.

5. Conclusions

In this paper it was demonstrated that the separation of basic solutes in CEC by the methodology proposed by Lurie et al. is quite well possible. This is in contrast to expectations. The conditions selected to separate basic solutes in CEC actually not only prove to overcome the anticipated limitations but actually turn the methodology into a powerful tool. The combination of two orthogonal separation mechanisms viz., partitioning and migration give rise to a much larger potential to manipulate the selectivity. The model derived for this case, describes separation

in a k' range from -1 to ∞ instead from 0 to ∞ in HPLC. Initial experiments have validated the retention model described in this paper.

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