Capillary Electrochromatography: Investigation of the Influence of Mobile Phase and Stationary Phase Properties on Electroosmotic Velocity, Retention, and Selectivity

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Abstract: The influence of mobile phase composition variation, organic solvent type, and the concentration of buffer salts on the magnitude of the electroosmotic flow (EOF) velocity, retention, and selectivity in capillary electrochromatography (CEC) has been investigated systematically. The observed change in EOF is explained in terms of change of solvent and stationary phase properties. These findings provide guidelines for the practitioner to select optimal conditions for CEC separations. On the other hand, it is demonstrated that stationary phase properties also have a profound effect on EOF velocity, solute retention, and selectivity of separation. It is demonstrated that the column packed bed of silica-based reversed-phase particles is the main contributor to EOF in CEC. Variation of stationary phases in CEC can be used in a similar way as in HPLC to improve the selectivity of separation of neutral substances. This also applies to the separation of weakly basic substances like triazines. © 1997 John Wiley & Sons, Inc. *J Micro Sep* **9**: 399–408, 1997

Key words: *capillary electrochromatography (CEC); electroosmotic flow (EOF); mobile phase properties; retention; selectivity; triazines; packed capillary column; fused silica; polyvinyl alcohol coating*

INTRODUCTION

Capillary electrochromatography (CEC) has become a feasible, new capillary separation technique combining attractive properties of (micro) high-performance liquid chromatography (HPLC), viz., simple control of retention and selectivity by mobile phase and stationary phase manipulation and of capillary electrophoresis (CE), viz., high efficiency of separation. In CEC the electroosmotic flow (EOF) is used as the mechanism to transport the mobile phase and the solutes to be separated through the column. This type of flow has favorable properties compared to hydraulic flow (or pressure-driven flow) used in an HPLC separation system. Flow velocity differences in the axial direction (illustrated in Figure 1) leading to band broadening by the so-called eddy diffusion are much smaller in electrical driven solvent transport than in HPLC. Moreover, EOF is generated by the packed bed of silica particles, which therefore does not provide an obstruction to flow, in contrast to pressure-driven solvent transport. This allows the usage of much smaller particles or longer separation columns than in (micro) HPLC. Work by Knox and other authors [1–14] has demonstrated that this potential can be achieved in practice. Figure 2 shows as an example the separation of polycyclic aromatic hydrocarbons (PAHs) on a 40-cm-long column packed with 3 μm Waters Spherisorb ODS1.

In CEC, separation is achieved by partitioning of the solute between mobile and stationary phase and, if the solutes are charged, also by differential electrophoretic mobilities of the solutes in the mobile phase. In the latter case the combined action of chromatography and electrophoresis has resulted in phenomenal zone compression of the separated solutes, tricyclic antidepressants [8]. Separation of neutral solutes and/or weakly basic and acidic solutes by CEC can be achieved with typical reversed-phase (RP) HPLC–like mobile and stationary phases with partitioning as the main retention mechanism. Therefore it should be straightforward, in principle, to transfer HPLC methods for such compounds directly to CEC, exploit the higher separation efficiency, and improve the method.

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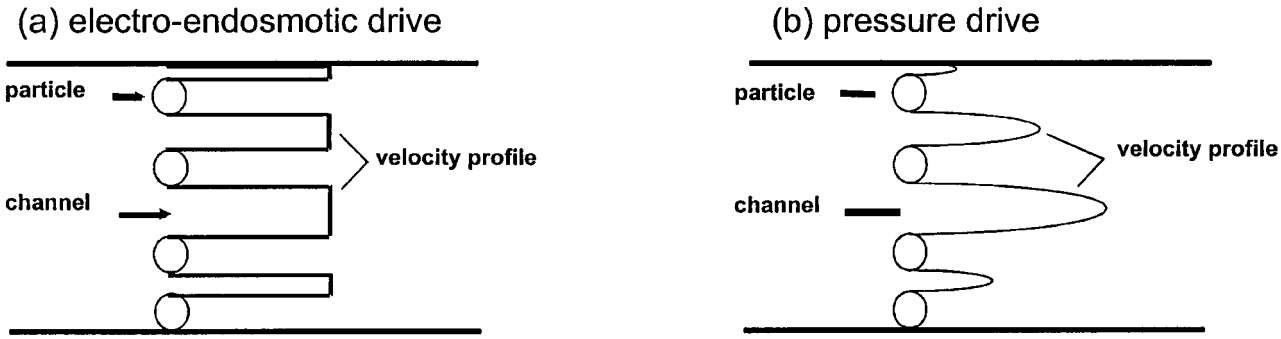

Figure 1. *Solvent velocity over the cross section of the column is constant when the solvent is driven by electrical force (a) in contrast with hydraulically driven solvents (b) [1, 2].*

In HPLC method development it is common to vary the mobile phase composition and/or the type or brand of RP stationary phase to optimize the separation. However, to date systematic investigations of the effect of mobile phase and stationary phase variation on retention and selectivity in CEC are absent. It was felt mandatory to investigate whether changes in mobile phase composition and stationary phase variation yield the same predictable effects on retention and selectivity in CEC as in HPLC. In CEC changes in the surface properties of the stationary phase, for example, will influence not only selectivity but also the EOF, as it is the packed bed itself that generates the flow. In this article the authors expand on their initial work in this area [14] and have tried to map the effect of mobile phase and stationary phase variation in a manner that will help chromatographers to select optimal separation conditions for CEC of neutral, weakly acidic, and basic substances.

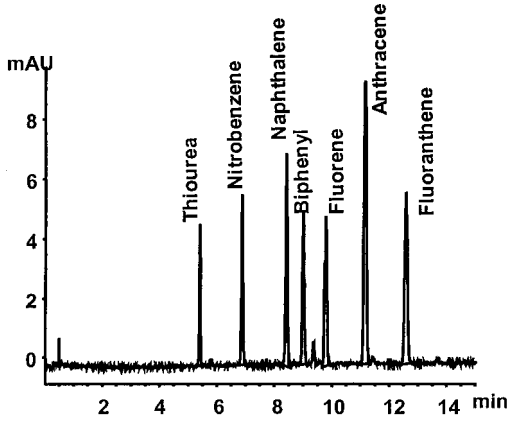

Figure 2. *Chromatogram showing that capillary electro chromatography is not constrained by the maximum pressure the HPLC pump can deliver. Smaller particles and longer columns can be used. Plate numbers of the individual peaks, 90,000–110,000. Conditions: column Waters Spherisorb ODS1, 3 μm, 400(485) × 0.1 mm; mobile phase acetonitrile/Tris HCl, 50 mM, pH 8, 80/20; voltage 30 kV; temperature 20°C. Other conditions: see experimental section.*

EXPERIMENTAL

Chemicals. The buffers used were trishydroxymethylaminomethane (Tris), 2-morpholinoethanesulfonic acid (MES), sodium acetate (NaOAc), and phosphoric acid (H_3PO_4) (all from E. Merck, Darmstadt, Germany). All buffers were adjusted to the desired pH using either HCl or NaOH (E. Merck). The solvents used were acetonitrile, methanol, and tetrahydrofuran (all from J. T. Baker B.V., Deventer, The Netherlands). The eluents were prepared by first adjusting the buffer to the desired pH, then mixing with the appropriate amount of organic modifier. In order to maintain a constant ion strength of the mobile phase with varying contents of organic modifier, the mobile phase was prepared from 10% aqueous buffer, $X\%$ organic solvent, and 90-$X\%$ water. The sample compounds were thiourea (E. Merck), methyl-4-hydroxybenzoate, ethyl-4-hydroxybenzoate, propyl-4-hydroxybenzoate, butyl-4-hydroxybenzoate (Fluka Chemie A.G., Buchs, Switzerland), pentyl-4-hydroxybenzoate and hexyl-4-hydroxybenzoate (synthesized in house), naphthalene (E. Merck), biphenyl (E. Merck), fluorene (Chem Service, Media, PA), anthracene (E. Merck), phenanthrene (Chem Service), and fluoranthene (Chem Service). The triazines were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Samples were prepared by mixing the appropriate buffer with a stock solution of ca. 20 mg/compound in 100 mL acetonitrile to the same acetonitrile/buffer ratio as the respective eluent.

Columns. The capillaries were packed according to a slurry packing procedure described in detail in LC-GC magazine [3]. Polyimide coated fused silica tubing was obtained from Polymicro Technologies

(Phoenix, AZ) with 100 μm i.d. and 350 μm o.d. Packed bed lengths of 25 and 40 cm were prepared; total column length was 8.5 cm plus packed bed length [indicated in all figures and text as 250(335) mm or 400(485) mm]. Packing materials were obtained from Hypersil (Runcorn, United Kingdom) (CEC Hypersil C18 3 μm, ODS Hypersil 3 μm, BDS–ODS Hypersil 3 μm, MOS–Hypersil 3 μm, and SCX/ODS–Hypersil 4 μm) and Phase Separations, Clwyd, United Kingdom (Waters Spherisorb ODS I 3 μm, Waters Spherisorb ODS II 3 μm and Waters Spherisorb C6/SCX).

Instrumentation. All CEC chromatograms were obtained with the Hewlett-Packard HP^{3D}CE (Hewlett-Packard GmbH, Waldbronn, Germany) instrument with the option to apply a pressure of 10–12 bars 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 bars to remove all air bubbles from the column. Samples were injected electrokinetically (5 kV for 3 s). The detection wavelength was at 250 nm with 80 nm bandwidth. High voltage was applied as a 3–6-s time ramp to avoid stress to the column.

RESULTS AND DISCUSSION

EOF, retention, and selectivity in CEC on silica-based C18 columns in dependence of the mobile phase composition. Variations of mobile phase composition, viz., percentage of organic modifier, type of organic modifier, pH of the buffer solution, and buffer concentration, are commonly used in HPLC to manipulate retention and selectivity of the separation. Because the solvent is driven by hydraulic force, the column pressure will change with physical properties, e.g., viscosity of the mobile phase. The hydraulic force generated by HPLC pumps in current instrumentation is adequate to maintain the desired solvent velocity.

In CEC the driving force is the electrical field along the length of the column. Electroosmotic flow occurs due to the presence of an electrical double layer on the surface of the particles in contact with an electrolyte giving rise to the zeta-potential. The flow velocity that is obtained is given by the Smoluchowski equation

$$u_{eo} = \frac{\varepsilon_0 \cdot \varepsilon_r \cdot \zeta \cdot E}{\eta} \quad (1)$$

where u_{eo} is the electroosmotic velocity, ε_0 is the permittivity of vacuum, ε_r is the dielectric constant of the mobile phase, ζ is the zeta-potential, and η is the viscosity of the mobile phase. A change of mobile phase composition will affect ε_r and η as well as the zeta-potential. For mixtures of an organic solvent with an aqueous buffer the ratio of dielectric constant and viscosity of the solvent will change, and it can be expected that the electroosmotic velocity will vary accordingly. Schwer and Kenndler have summarized these data to predict the magnitude of EOF in Capillary Zone Electrophoresis (CZE) [15]. The ratio of ε_r/η vs. percent organic modifier at 25°C for methanol/water and acetonitrile/water is given in Figure 3. From these data it is expected that the electroosmotic velocity will decrease with reduction of acetonitrile or methanol concentration in the mobile phase through a minimum and will increase again on low percentage of these organic modifiers.

In this work the EOF velocity at 20°C as a function of percent organic modifier was determined in a packed capillary [250(335) × 0.1 mm packed with CEC–Hypersil C18] with acetonitrile and methanol as organic modifiers: The mobile phase was prepared such that a constant ion strength of the mobile phase mixture was obtained. This is necessary as a change in overall ion strength will affect the zeta-potential and therefore obscure the observations. The mobility of the eluent is calculated from the elution time of thiourea, which is assumed to be nonretained at all compositions tested. By multiplication of the eluent mobility with the field strength (in volts per centimeter) the electroosmotic flow velocity is obtained directly.

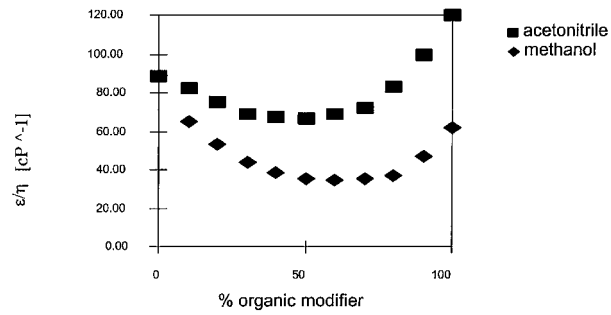

Figure 3. *Plot of the ratio dielectric constant ε over viscosity η for MeOH/water and ACN/water. Data from Schwer and Kenndler [15].*

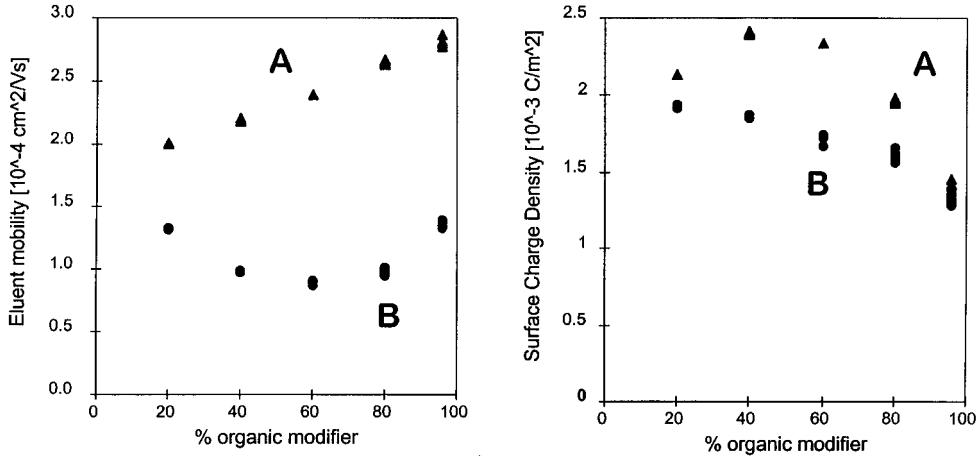

Figure 4. *Plot of eluent mobility (left) and electrophoretic charge density (right) versus percentage acetonitrile (a) and methanol (b) in the mobile phase. Column: CEC–Hypersil C18, 3μm, 250(335) × 0.1 mm, mobile phase x% organic modifier, (100 − 4 − x)% water, 4% 25 mM Tris–HCl, pH 8, temperature 20°C, pressure on both vials 10 bar; t_0, marker thiourea.*

Figure 4(a) shows the eluent mobilities for the acetonitrile/buffer and methanol/buffer mixtures. While the mobilities for the methanol system show qualitatively the behavior expected from the ε_r/η data, the mobilities of the acetonitrile system show a steady increase with increasing acetonitrile content. This behavior of the acetonitrile/buffer system has also been observed by other authors [5, 6]. This observation leads to the conclusion that, in addition to the change of the ratio of dielectric constant and viscosity, the zeta-potential and thus the charge density of the particle surface in the packed bed are changing with organic modifier content.

The electrokinetic charge density, i.e., the charge density at the surface of shear, was calculated using the Gouy–Chapman theory [16].

For small surface potentials (up to ca. 40 mV) the electrokinetic charge density σ_e can be expressed as [16]

$$\sigma_e = \varepsilon_0 \varepsilon_r \kappa \zeta \tag{2}$$

where κ is the Debye–Hückel parameter

$$\kappa = \left[\frac{2cF^2}{\varepsilon_0 \varepsilon_r RT} \right]^{1/2} \tag{3}$$

where c is the concentration of electrolyte, F the Faraday constant, R the gas constant, and T temperature.

The σ_e values calculated from the ζ-potential are shown in Figure 4(b). One has to be aware of the fact that these charge densities represent an apparent charge density, i.e., the charge density that actually contributes to the EOF. Figure 4 shows that σ_e changes with organic modifier content. This effect is more pronounced in the acetonitrile system than in the methanol system. At high organic modifier content the apparent charge densities are the same for methanol/buffer and acetonitrile/buffer. Here the differences in EOF have to be attributed solely to the difference in ε_r/η. At an organic modifier content of 60%, however, σ_e is 35% higher in the acetonitrile system than in the methanol system. The reasons for these changes in apparent charge density need yet to be elucidated. Possible causes can be changes in adsorption of the organic compound at the surface or changes in conformation of the C18 chains influencing the electrical double layer [17].

Selectivity of separation is affected by the type of organic modifier used. Exchange of acetonitrile for methanol or tetrahydrofuran is common practice in HPLC. In the preceding section it was shown that in CEC the magnitude of EOF varies with the type of organic modifier. Figure 5 shows as an example the separation of a test mixture consisting of alkyl 4-hydroxybenzoic acid esters (alkyl parabens) and PAHs with acetonitrile (top trace), methanol (middle trace), and tetrahydrofuran (lower trace) as the organic modifier. The column used was a 25 cm CEC–Hypersil C18, 3 μm. Mobile phases contained 80% organic modifier and 20% of 25 mM Tris buffer adjusted to pH 8 prior to mixing. As can be expected, the capacity ratio of the solutes with 80% methanol is higher because this solvent has lower elution strength than acetonitrile. But in accordance with the observations in Figure 4, the electroosmotic velocity with methanol as modifier has decreased by

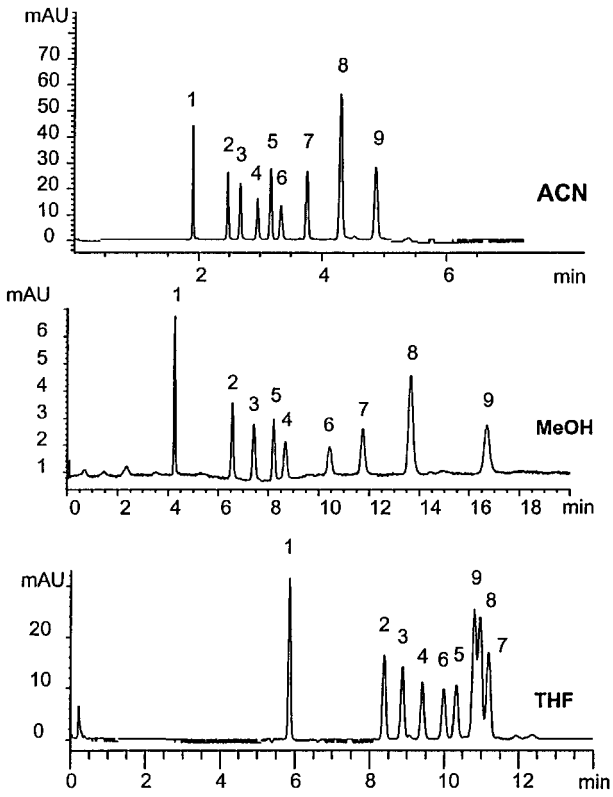

Figure 5. *Effect of the type of organic modifier on EOF, retention, and selectivity of neutral substances in CEC. Column: CEC–Hypersil C18, 3 μm, 250(335) × 0.1 mm, mobile phase 80% organic modifier / 20% 25 mM Tris · HCl, temperature 40°C, voltage 25 kV. Sample constituents: (1) thiourea, (2) butylparaben, (3) pentylparaben, (4) hexylparaben, (5) naphthalene, (6) heptylparaben, (7) fluorene, (8) anthracene, (9) fluoranthene. Top trace with acetonitrile, middle trace with methanol, and lower trace with tetrahydrofuran as organic modifier.*

a factor of ca. 2.4. A selectivity change is observed also; naphthalene, peak 5 elutes before hexylparaben, peak 4. This change also occurs in HPLC, as was verified in a separate experiment. In the lower trace, 80% tetrahydrofuran has been used as organic modifier. Solute retention is lower with tetrahydrofuran, as expected. But it is striking to see that the EOF decreases by a factor of 3 with tetrahydrofuran compared to acetonitrile. Values for dielectric constant and viscosity of the mixture water/tetrahydrofuran were not available. But estimated from the values of the dielectric constant and viscosity of the pure organic solvents, a decrease by a factor of 1.5 is expected. The deviation is attributed to a substantial change in accessibility of the surface silanol with tetrahydrofuran containing solvents. Selectivity of separation changes dramatically with tetrahydrofuran as modifier, illustrated by reversal of elution order of solutes 7, 8, and 9 in addition to the retention order reversal of solutes 5 and 6. Again it was confirmed that these selectivity changes are the same as in HPLC and are not a property of CEC.

The influence of the buffer ion strength on the magnitude of EOF in CEC was investigated by variation of the Tris · HCl concentration in the eluent. Buffers containing 5–100 mM Tris · HCl at pH 8 were mixed with 80% acetonitrile, thus the final ion concentration in the eluent was between 1 and 100 mM. Thiourea was used as a nonretained solute to measure t_0 and to calculate the eluent mobility. The measurements were done on a 250(335) × 0.1 mm capillary column packed with CEC–Hypersil C18, 3 μm, at 20, 30, and 40°C. Results are given in Figure 6.

According to theory [1], the EOF will increase with a decrease of buffer concentration. With decreasing ion strength the thickness of the diffuse double-layer length increases, leading to an increase in zeta-potential. The increase of EOF with temperature is mainly due to the increase in the ε/η ratio, although the temperature change will also affect the zeta-potential. Thus to achieve high EOF in packed column CEC, it is recommended to work at low buffer concentrations and above ambient temperature.

Dependence of EOF, retention, and selectivity on the type of stationary phase in CEC. In a previous publication [14] the influence of stationary phase type on EOF has been investigated (Figure 7). The

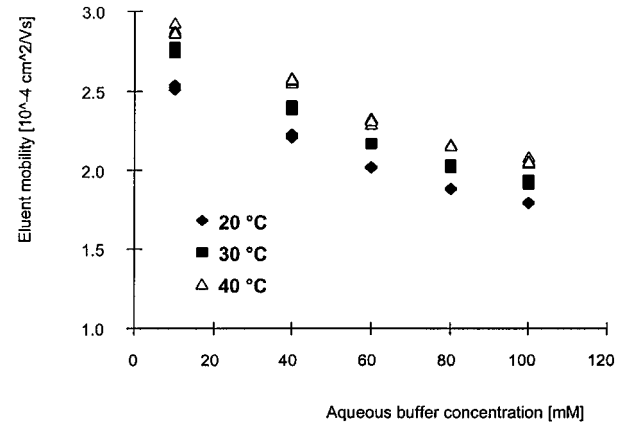

Figure 6. *Effect of buffer concentration and temperature on EOF in CEC. Column CEC-Hypersil C18, 3 μm, 250(350) × 0.1 mm; mobile phase 80% acetonitrile / Tris–HCl, pH 8, buffer concentration and temperature given in the figure; voltage 20 kV; dead-time marker, thiourea.*

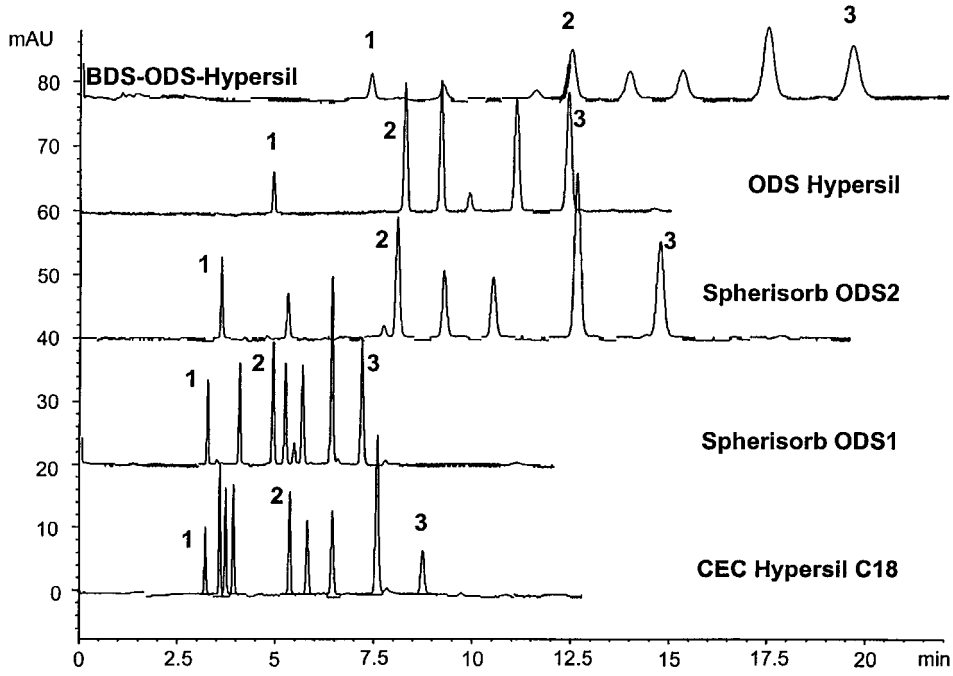

Figure 7. *Separation of PAHs on five reversed-phase C18 stationary phases. Column 250(335) mm × 0.1 mm, 3 μm, mobile phase 80% acetonitrile/20% 50 mM Tris–HCl, pH 8, 20 kV, temperature 20°C, 10 bar pressure applied to both ends of capillary, 20°C. Samples were not identical but all contained thiourea (1), napthalene (2), and fluoranthene (3).*

separation of a test mixture of neutral solutes on five different silica-based reversed phases with the same mobile phase, temperature, and field strength is shown in this figure. This work has clearly demonstrated that silica-based RP stationary phases with a high surface concentration of silanol groups show high EOF whereas phases with low surface concentration of silanol groups (like "base-deactivated" silica reversed phase, BDS–ODS–Hypersil) show low EOF. Unpublished results with other base-deactivated stationary phases gave similar results. This finding has led the authors to the postulation that, in CEC, the packed bed of stationary phase is the main contributor to EOF.

On the other hand, Horváth et al. presented arguments for a substantial contribution to EOF in packed bed CEC generated by the capillary wall [18]. In order to clarify this question, according to a procedure by Schomburg et al. [19], fused silica capillaries coated with polyvinylalcohol (PVA) were packed with CEC–Hypersil C18 by the process described in the experimental section. The PVA coating effectively shields the silanol groups on the silica surface of the capillary tube and therefore eliminates a contribution of EOF from the capillary wall. In a separate experiment, the EOF in such a coated capillary was measured under the conditions of the CEC separation (Figure 8) and was found to be ca. 0.4 mm/s. This is a negligible EOF compared to the EOF in the packed capillary (1.6 mm/s) and in an open fused silica capillary under these conditions (2.4 mm/s).

A test mixture of alkyl parabens and polyaromatic solutes were separated on this column and the result compared to the separation of this sample on a column packed with an uncoated fused silica capillary. The result is shown in Figure 8. From the elution times of all peaks on both columns, one can conclude that there is no significant change in EOF or in retention as a consequence of the PVA wall coating. In separate experiments it was verified that the PVA coating has not been removed from the capillary wall during the column packing process or by the organic solvent in the mobile phase. Taking all these findings into account leads to the conclusion that in packed column CEC the EOF is almost exclusively generated by the particles.

Evaluation of mixed-mode stationary phases for CEC. In an earlier publication, the authors investigated the influence of the mobile phase pH on the magnitude of the electroosmotic velocity in CEC [14]. As anticipated, the EOF decreased with lower pH in the mobile phase for different silica-based RP-type packings because the ionization of the sur-

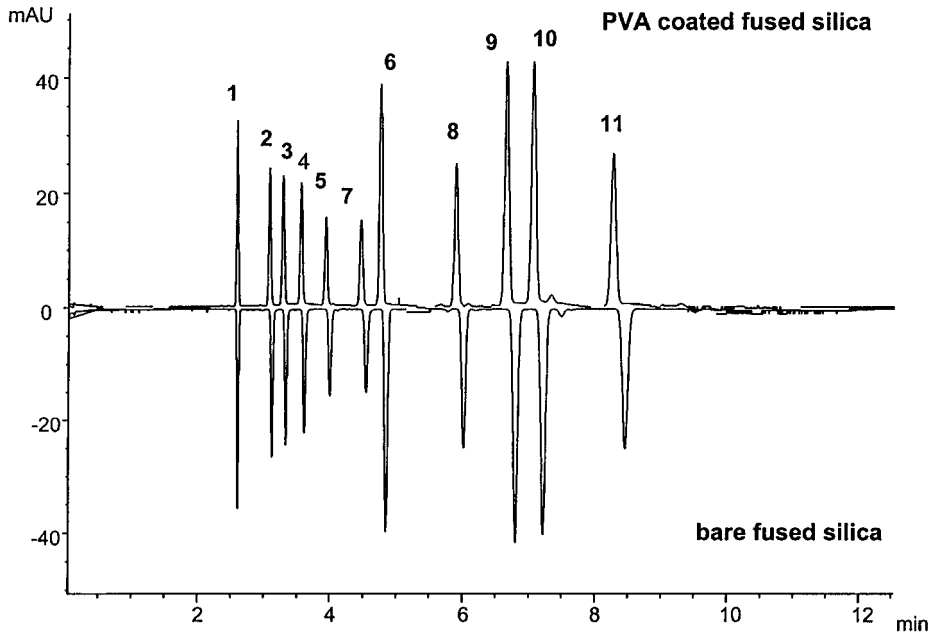

Figure 8. *Comparison of separation of the neutral test mix on a CEC column packed with CEC–Hypersil C18 in a polyvinylalcohol-coated fused silica capillary (top trace) and packed in a bare fused silica capillary (mirror trace). Column 250(335) × 0.1 mm, mobile phase 80% acetonitrile/20% 25 mM Tris–HCl, pH 8, temperature 20°C. Solutes: (1) thiourea, (2) ethylparaben, (3) propylparaben, (4) butylparaben, (5) pentylparaben, (6) naphthalene, (7) hexylparaben, (8) fluorene, (9) anthracene, (10) anthracene, (11) fluoranthene.*

face silanol groups was suppressed. In the practice of HPLC, though, one wants to be able to vary the pH of the mobile phase in order to optimize the separation. It is thus undesirable in CEC that the mobile phase velocity decreases when the pH of the mobile phase is lowered. For that reason, mixed-mode stationary phases that have both C18 alkyl chains and strong cation exchange groups, propyl sulfonic acid, attached to the surface have been prepared by stationary phase manufacturers. A strong cation exchange group has a permanent negative charge even at low pH values. Therefore such phases are expected to maintain a stable EOF over a broad pH range. As an example of such a phase, the SCX/ODS Hypersil from Hypersil (Runcorn, United Kingdom) was packed in FS capillaries and tested with a neutral solute sample (Figure 9).

In Figure 9(a) the chromatograms at pH 4, 6, and 8 are shown. In all cases the pH value refers to the pH of the aqueous buffer prior to mixing with acetonitrile. As can be seen from the elution time of the first peak, thiourea, there is a slight decrease of the EOF with a decrease of pH. In Figure 9(b) the eluent mobilities on the C18/SCX mixed-mode phase are compared to those on CEC–Hypersil C18. The EOF of the mixed-mode phase increased due to the presence of the negative sulfonic acid groups. However, still a decrease was observed at lower pH. This is attributed to the contribution to EOF by the native surface silanol groups which decreases at low pH. This result indicates that in this mixed-mode phase the silanol groups have a substantial contribution to the generation of flow.

An additional mixed-mode phase was examined in this work, viz., C6/SCX from Waters Phase Separations. This phase contained propyl sulfonic acid group bonded onto Waters Spherisorb (8 nm pore, 180 m^2/g surface area, and 0.45 mL/g pore volume). The propyl sulfonic acid silane was reacted in non-stoichiometric quantities followed by bonding with a monochloro C6 silane [20]. It was expected that a 50/50 coverage of the sulfonic acid and the C6 is obtained in this way. Columns were prepared with this stationary phase by the process described above. A third stationary phase, a C8 MOS–Hypersil, 3 μm, was examined as it is expected that C8-type RP packings may also show high EOF because accessibility of surface silanol groups would be easier with less hydrophobic coatings. All columns were tested with the 10 component sample mix of alkyl parabens and aromatics with thiourea added as t_0 time marker. Also the three stationary phases, CEC-Hypersil C18, MOS–Hypersil, and C6/SCX Waters Spherisorb, were compared in the separation of a mixture of triazines like atrazine, cyanazine, simazine, etc. The results are shown in Figures 10 and 11.

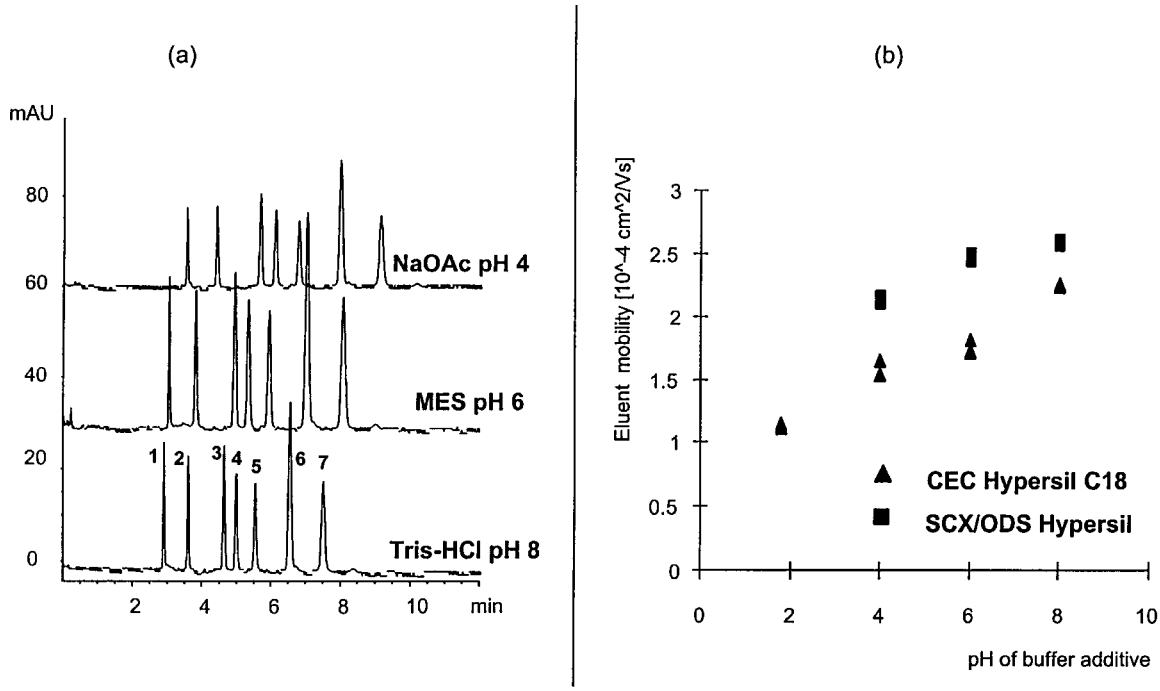

Figure 9. *Separation of PAHs on SCX / ODS Hypersil at three different pH values. Column 250(350) × 0.1 mm, particle size 4 μm, mobile phase 80% acetonitrile / 20% 50 mM buffer (Tris · HCl, pH 8; MES, pH 6; NaOAc, pH 4), temperature 20°C; voltage: 20 kV; pressure on both vials 10 bars. Solutes as for Figure 2.*

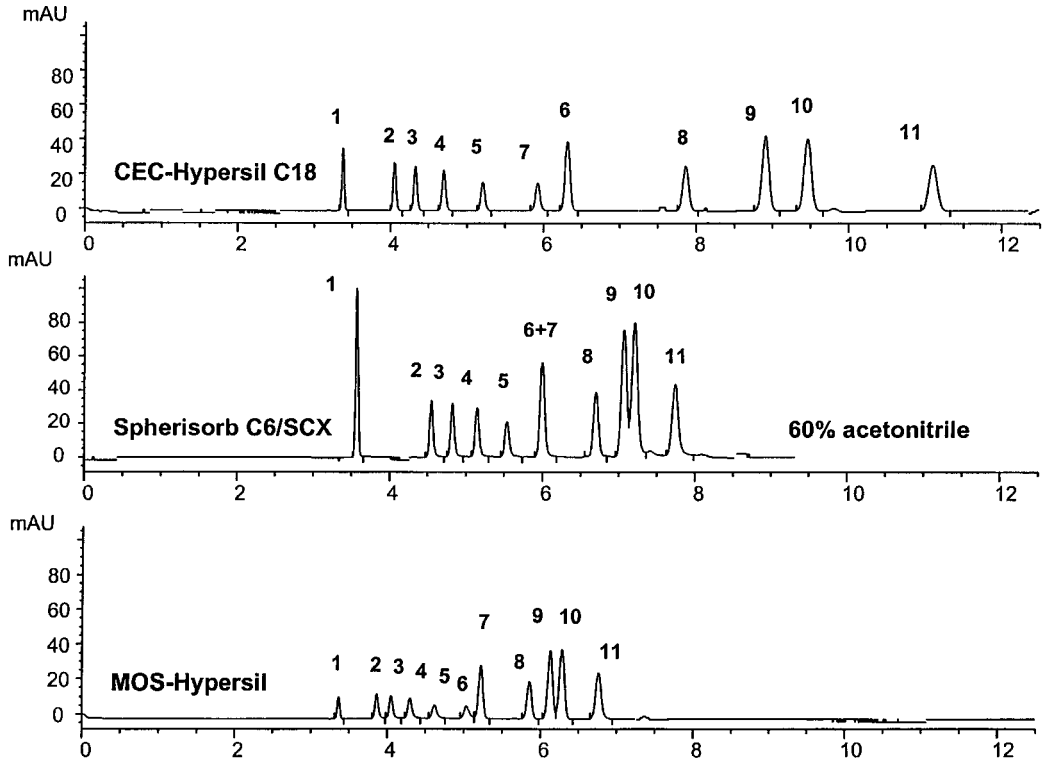

Figure 10. *CEC separation of the test mixture of neutral solutes on three different stationary phases. All columns 250(335) × 0.1 mm; particle size: MOS−Hypersil, CEC−Hypersil C18, 3 μm, C6 / SCX Waters Spherisorb, 4 μm. Mobile phase 80% acetonitrile / 20% 25 mM Tris · HCl, pH 8.0, on the C6 / SCX Waters Spherisorb, 60% acetonitrile / 40% buffer, temperature 20°C, voltage 20 kV. Solutes are the same as in Figure 8.*

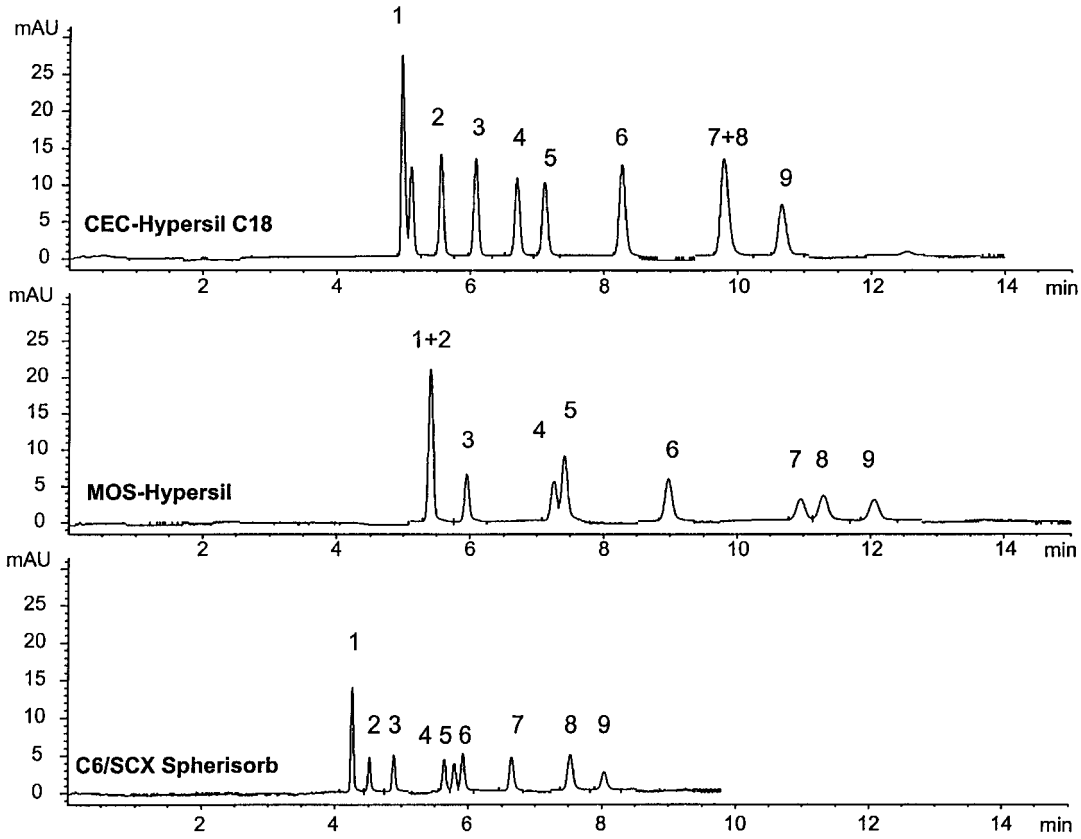

Figure 11. *CEC separation of a mixture of triazines on three different stationary phases. All columns as in Figure 10. Mobile phase 50% acetonitrile/50% 25 mM NaOAc, pH 8, temperature 20°C, voltage 25 kV. Sample constituents dissolved in acetone:* (*1*) *acetone + desisopropylatrazine,* (*2*) *desethylatrazine,* (*3*) *simazine,* (*4*) *cyanazine,* (*5*) *desbutylterbutylazine,* (*6*) *atrazine,* (*7*) *sebutylazine,* (*8*) *propazine,* (*9*) *terbutylazine.*

As can be expected, retention of the neutral test solutes is lower on the C6/SCX Waters Spherisorb and on the MOS–Hypersil (Figure 10). In the C6/SCX column, the acetonitrile content was reduced to 60% in order to retain the solutes. Slight selectivity changes are observed, e.g., reversal of retention order of naphthalene and heptylparaben. The separation of the triazine mixture on the three phases gave good peak shapes for all solutes (Figure 11). However, interesting selectivity changes for the solutes were found. For example, sebutylazine and propazine are not separated on the CEC–Hypersil but are separated by CEC on the C6/SCX and on the MOS–Hypersil.

CONCLUSIONS

In capillary electrochromatography, the EOF depends on mobile phase and stationary phase properties. Therefore in case one wants to optimize a separation by variation of mobile phase composition, one has to be well aware of the impact these changes have on the magnitude of the EOF. The changes of EOF with solvent properties (dielectric constant, viscosity, ion strength, and temperature) are within certain predictable limits. The influence on EOF of changes in surface properties of the stationary phase (also those induced by changing the mobile phase) is less well predictable. Further studies in this area are required.

Our results have shown that the stationary phase bed is the main contributor to the EOF in a packed capillary. Further studies in this field are important to fully unravel the mechanism of EOF generation in a packed bed.

As in HPLC, variation of stationary phases is an important tool to achieve/optimize separation. It can be anticipated that CEC will eventually become a widespread technique when the diversity of stationary phases available in the CEC format becomes as large as in HPLC.

Capillary electrochromatography currently is a good separation technique for neutral, weakly basic (triazines) and weakly acidic (alkyl parabens) compounds. The separation of strongly acidic and basic

substances by CEC still poses a problem, as the highly inert (base-deactivated) stationary phases used for separation of these compounds show virtually no EOF. More research is required in the field of stationary phase and method development to make CEC feasible for the separation of permanently charged compounds.

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