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Influence of mobile phase composition on electroosmotic flow velocity, solute retention and column efficiency in open-tubular reversed-phase capillary electrochromatography

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Abstract

The effects of some experimental parameters, such as the volume fraction and type of organic modifier in the mobile phase, and the concentration, type and pH of the buffer on the electroosmotic flow velocity, the retention behavior of test solutes, and the column efficiency have been investigated in capillary electrochromatography (CEC) using an open-tubular column of 9.60 μm I.D. with a porous silica layer chemically modified with C_{18} as stationary phase. The retention of a group of polycyclic aromatic hydrocarbons (PAHs) used as a test mixture varied significantly by changing the organic modifier content in the hydroorganic mobile phase according to the reversed-phase-like selectivity of the stationary phase. In addition, an increase in the percentage of organic modifier resulted in a slight increase in the linear velocity of the EOF. On the other hand, when the phosphate buffer concentration was increased over the range 1–50 m*M*, the electroosmotic mobility fell dramatically, the retention of the solutes decreased steadily, and the plate height showed a significant increase. The results obtained with phosphate, trishydroxymethylaminomethane or 2-morpholinoethanesulfonic acid as buffers were similar when pH remained constant. Optimization in CEC was essential to achieve further enhancement of separation performance, because the analysis time and separation resolution are essentially affected when varying operating parameters. Separations of seven PAHs with more than 100 000 plates are presented within 4 min analysis time.

Keywords: Electrochromatography; Mobile phase composition; Electroosmotic flow; Efficiency; Open-tubular electrochromatography; Polynuclear aromatic hydrocarbons

1. Introduction

The first open-tubular liquid chromatography (OT-LC) experiments were described 20 years ago [1,2], and although this technique has shown a great potential to reach high efficiencies [3,4], at present, it is used only in university laboratories and research institutes. Commercial OT-LC instruments are still not available due to the experimental difficulties in efficiently operating with the extremely small dimensions of the system. In contrast, several commercial instruments have been developed for capillary electroseparation techniques since the introduction of capillary zone electrophoresis, around 1980 [5].

Capillary electrochromatography (CEC) gained popularity during the 1990s [6], although it was originally used in the 1970s [7] and developed 7 years later [6]. This technique combines the desirable features of both high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE),

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but CEC is still relatively undeveloped compared to a more mature method such as HPLC and progress in column and instrument technology is changing rapidly [8,9].

CEC uses an electroosmotic flow (EOF) to transport the mobile phase and solutes through the chromatographic column instead of the hydraulic flow (head pressure as the driving force) that occurs in HPLC. The flow velocity obtained is given by the Smoluchowski equation [10]:

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

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, η is the viscosity of the mobile phase, V is the voltage applied across the column, L_t is the total length of the column, μ_{eo} is the electroosmotic mobility and E the electric field strength. A change of the mobile phase composition will affect ε_r and η as well as ζ [11]. On the other hand, this flow velocity also depends on the ionic strength of the electrolyte (I, mol/l) through the electroosmotic mobility in accordance with [12]

$$\mu_{eo} = \frac{\sigma}{\eta} \cdot \left[\frac{\varepsilon_0 \varepsilon_r R T}{2 F^2} \cdot \frac{1}{I} \right]^{1/2} \quad (2)$$

where σ is the charge density of the excess ions in the Gouy–Chapman layer, R is the gas constant, T is the temperature, and F is the Faraday constant.

The separation process is based on differential partition between two phases, differential electromigration, or a combination of these two. This approach can be used for tuning the selectivity allowing to obtain unique selectivities. The mobile phases are similar to those used in conventional reversed-phase HPLC, a hydro–organic mobile phase with acetonitrile or methanol as the most widely used organic modifiers, except that a dilute buffer is added to ensure sufficient electrical conductivity. The parameters determining the performance of CEC with packed capillary columns (PCCs) have been investigated [13] and the effects of mobile phase pH and the addition of organic solvents on the chromatographic behaviour have been studied [14]. The value of u_{eo} in PCCs depends on the amount of surface charges, pH, concentration of electrolyte in the mobile phase, and composition of the mobile phase [12,15,16]. However, a systematic investigation of the effect of mobile phase composition on separation in CEC using an open-tubular format has not been carried out yet.

In this paper, the open-tubular approach to CEC is used to study systematically the influence of the variation in the mobile phase composition on the EOF velocity, the retention behaviour of neutral solutes, and column efficiency. Thus, changes in the concentration, type and pH of the buffer, as well as in the volume fraction and nature of the organic modifier, were performed to find out whether the mobile phase composition yields the same predictable effects on reversed-phase separation in open-tubular (OT) CEC as in packed CEC or HPLC, in order to prove that this format is a feasible alternative to packed columns. Optimal conditions are selected for separation of neutral compounds by OT-CEC.

2. Experimental

2.1. Instrumentation

The OT-CEC experiments were performed in a laboratory-assembled system for CE. The electrical field was supplied with a 0–30 kV high-voltage power supply from Glassman High Voltage (Model EH30P03; Whitehouse Station, NJ, USA). Platinum electrodes were used to connect the power supply to the buffer reservoirs, located at each end of the capillary. Detection was carried out at the cathodic side using an UV variable-wavelength detector with on-column cell (Model 200; Linear Instrument, Reno, NV, USA). Electrochromatographic separations were performed at room temperature, and electrochromatograms were recorded with an acquisition data system Model Star 4.5 from Varian Associates (Sugar Land, TX, USA) via a personal computer. A 654 pH meter from Metrohm (Herisau, Switzerland) was used to adjust pH of the buffers.

2.2. Materials and chemicals

Fused-silica capillaries of nominal 10 μm I.D.×

365 μm O.D. were purchased from Polymicro Technologies (Phoenix, AZ, USA).

Chemicals used were of analytical grade purity or higher and used as received. Potassium dihydrogenphosphate and sodium hydroxide were purchased from Panreac (Barcelona, Spain), and trishydroxymethylaminomethane (Tris) and 2-morpholinoethanesulfonic acid (MES) were purchased from Merck (Darmstadt, Germany). The solvents methanol (MeOH) and acetonitrile (MeCN) were HPLC-grade and purchased from Scharlau (Barcelona, Spain). Water was obtained from a Millipore Milli-Q system (Bedford, MA, USA). Dimethylformamide (DMF), naphthalene, acenaphthene, fluorene, phenanthrene, anthracene and pyrene were obtained from Merck, and acenaphthylene and fluoranthene were obtained from Aldrich (Milwaukee, WI, USA).

2.3. Preparation, purge and equilibration of the open-tubular column

The open-tubular column (OTC) used in this work has 9.60 μm of inner open diameter and it was prepared according to a previous work [3]. This column has a thin porous silica layer of 0.70 μm with C_{18} groups chemically bonded onto the prepared layer. A small section of the polyimide coating of the OTC was removed to provide an optical window for in-column UV detection [17]. The OTC is filled with the mobile phase by applying pneumatic pressure once placed in the instrument (purging the capillary for 10 min after the emergence of any air). The capillary is then equilibrated for 20 min, at low voltage for 15 min, before the voltage is increased to the desired value. The mobile phase in the reservoirs must be replaced after each analysis to improve retention time reproducibility and to achieve good baselines in the separations. Before shut-down the capillary was flushed with the organic modifier of the mobile phase and stored overnight.

2.4. Mobile phase and sample preparation

Buffers used were phosphate, Tris, and MES. All of them were adjusted to the desired pH using 0.1 *M* NaOH. The organic modifiers used were MeCN and MeOH. The mobile phases were prepared by first adjusting the buffer to desired pH, then mixing with the appropriate amount of organic modifier. All mobile phases were degassed by purging with He for 2 min, and filtered with 0.45 μm nylon syringe filters from Scientific Resources (Eatontown, NJ, USA).

Stock solutions of the individual PAHs were first prepared at a concentration of 2.0–4.0 mg/ml in pure MeOH due to their low solubility in water. Test samples were prepared by mixing the appropriate volumes of each stock solution with methanol [concentration of each polycyclic aromatic hydrocarbon (PAH) in the test sample was 0.1–0.5 mg/ml], and injected hydrodynamically by siphoning. PAHs were detected by UV absorbance at 220 nm or 235 nm depending on the mixture.

3. Results and discussion

In order to gain a better understanding of the fundamental parameters which control the chromatographic performance in OT-CEC, a systematic study was performed using a neutral test mixture containing DMF as EOF marker, and the following PAHs: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene. The test sample was prepared in MeOH, a solvent stronger than the mobile phase (hydro–organic mixture), because relatively high concentrations of solutes were required for easy UV detection. In spite of the fact that the sample solvent was a stronger eluent than the mobile phase, no zone defocusing was observed. The use of a solvent for injection with weaker elution strength than the mobile phase should result in a focusing effect [18]. However, in this case, distorted peak shapes (peak tailing and significantly broadened) were obtained. This result could be due to the low solubility of the solutes in the mobile phase which had a higher content in water.

On the other hand, injection of the samples was done hydrodynamically by siphoning, obtained by elevating the injection reservoir relative to the exit reservoir (Δh_i) during the injection time (t_i). The length of the sample loaded is a function of the column dimensions (total length, L_t, and inner diameter, d_c), the density (ρ) of the sample solution, and the viscosity (η) of the mobile phase, according to the following equation [19]:

$$L_{inj} = \frac{\rho g d_c^2}{32\eta L_t} \cdot t_i \cdot \Delta h_i \quad (3)$$

where g is the gravitational constant (9.8 m/s^2). With this injection technique the length of the sample loaded is dependent of the viscosity of the mobile phase ($\eta_{65\%\ MeOH} = 1.44$ and $\eta_{65\%\ MeCN} = 0.65$, ratio 2.2), i.e., of its composition. Thus, it is necessary to confirm that the extra-column effect due to the injection process can be neglected for all the mobile phases tested. With this aim, a study of the impact of the injected sample length on the efficiency of the separation was performed by varying the injection parameters ($\Delta h_i \times t_i$) using the mobile phase with the smallest viscosity (65% MeCN in 1 m*M* phosphate buffer) [20]. The results showed that to avoid a significant efficiency loss (>10%) injections obtained by elevating the injection reservoir 15 cm relative to the exit reservoir for 10 s were needed, which corresponds to an injected sample length of about 0.2 mm. Therefore, according to Eq. (3) when mobile phases of larger viscosity (e.g., with phosphate buffer more concentrated or with percentages of MeCN between 50 and 65% or with MeOH) are used, the length of the sample loaded will be smaller than 0.2 mm if a hydrodynamic injection by siphoning with a height difference of 15 cm between both vials for 15 s is done. In addition, the repeatability of the injection process was acceptable, with a relative standard deviation ($RSD_{n=5}$) less than 6% for both peak area (proportional to the injection volume) and efficiency.

3.1. Effect of buffer concentration on electroosmotic mobility, solute retention, and efficiency

The magnitude of the electroosmotic mobility is one of the most important parameters for predicting the accessible linear velocity in CEC with chemically modified capillaries under reversed-phase conditions. Theoretical considerations concerning the effect of ionic strength of the electrolyte in CEC indicate that in absence of thermal and double layer overlap effects, the electroosmotic mobility should decrease steadily when increasing electrolyte concentration, and that column efficiency is essentially unaffected when varying the electrolyte concentration over a reasonable range [21]. The effects of the buffer concentration on the EOF, the retention of the solutes, and the efficiency were determined for PCCs [12,22]. However, the dependence of these parameters upon buffer concentration when an OTC is used in CEC has not been described yet. Thus, separations with a OTC-C_{18} of 9.60 μm I.D. were performed at fixed MeCN concentration of 60% in the mobile phase and pH 7.0 aqueous phosphate buffer of variable concentrations (1–50 m*M*). Examination of the electrochromatograms shown in Fig. 1, where the first eluting peak (DMF) was used as EOF marker, clearly reveal how the EOF decreased when increasing the buffer concentration.

Table 1 summarizes the EOF velocity (u_{eo}),

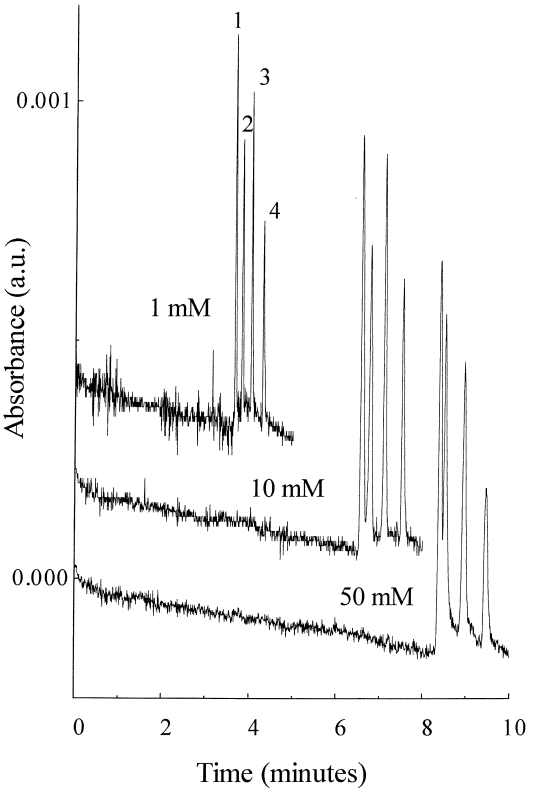

Fig. 1. Effect of phosphate buffer concentration on the separation of four test compounds by OT-CEC. Experimental conditions: OTC, 41 cm (31 cm to the detector)×9.60 μm I.D.; mobile phase, MeCN–phosphate buffer pH 7.0 (60:40); hydrodynamic injection, 15 cm for 15 s; applied voltage, 20 kV; detection, UV at 220 nm. Test compounds were (1) DMF, (2) naphthalene, (3) phenanthrene and (4) pyrene.

Table 1
Effect of the phosphate buffer concentration on the electroosmotic mobility (μ_{eo}), the linear velocity (u_{eo}; $RSD_{n=5}=0.5–0.9\%$), the retention (k; $RSD_{n=5}=1.7–4.3\%$), the plate height (H; $RSD_{n=5}=1.8–3.2\%$), and the elution time (t_r; $RSD_{n=5}=0.5–0.9\%$). Experimental conditions as stated in Fig. 1

Phosphate (mM)	u_{eo} (cm/s)	μ_{eo} ($\times 10^{-4}$ cm^2/V s)	Pyrene		
			k	H (μm)	t_r (min)
1	0.145	2.98	0.17	2.1	4.4
10	0.081	1.67	0.15	3.5	7.6
50	0.064	1.32	0.13	4.6	9.5

electroosmotic mobility (μ_{eo}), retention factor (k) and efficiency (H) data collected from the electrochromatograms in Fig. 1. From these data, it is possible to demonstrate that the electroosmotic mobility has an inverse relationship with the square root of the buffer concentration in the range 1–50 mM ($y = 1.933x + 1.049$ with $r^2 = 0.9995$, number of points = 15) according to Eq. (2). On the other hand, a large deviation from the predicted trends was observed in the case of efficiency, because the plate height showed a significant increase (>100%) when buffer concentration increased over the range 1–50 mM. Although the mobile phase velocity was not kept constant, the impact of this effect on efficiency was minimal, only an efficiency loss smaller than 20% was observed when the k value was not higher than 0.2 [20]. Finally, the retention of the solutes falls off steadily when increasing the buffer concentration. For practical purposes, substantial EOF could be generated in the absence of an electrolyte in the mobile phase but poor migration time reproducibility was obtained (RSD>5%). It can be concluded from these data that to work at a low buffer concentration (1 mM) is recommended since it provided the best efficiency and retention, i.e. the highest resolution with the smallest separation time.

3.2. Effect of the nature and pH of the buffer

In addition to phosphate, MES and Tris were evaluated as buffers in the mobile phase. These zwitterionic buffers generate much lower currents than the inorganic buffers such as phosphate, but this characteristic is not important when OTCs of about 10 μm I.D. are used in CEC, because a very reduced current is generated in these electrochromatographic systems (only a few μA or less). However, the organic nature of MES and Tris could improve the solubility of organic compounds such PAHs in the separation buffer increasing the efficiency.

The electrochromatograms obtained with the different buffers at the same pH show that no significant effects on the separation were observed (see Fig. 2). In addition, examination of the electrochromatograms shown in Fig. 2 clearly reveals the excellent reproducibility obtained in the separations performed with different mobile phases and in different days. On the other hand, when each buffer

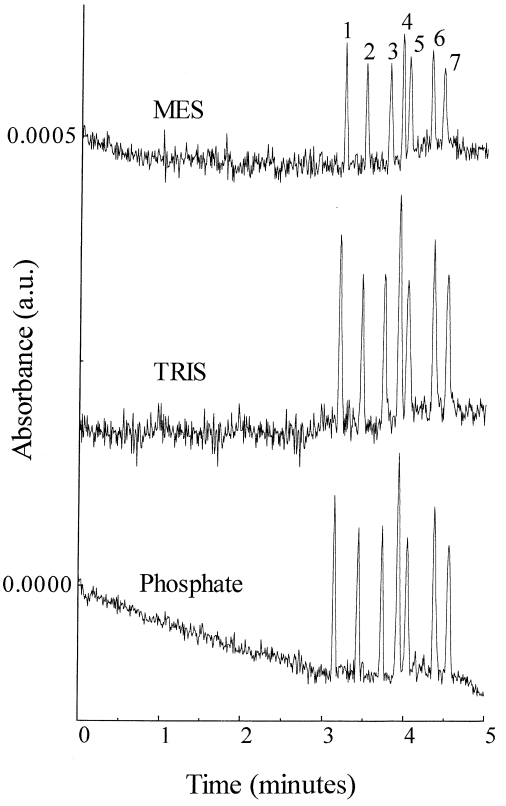

Fig. 2. Separation of a test mixture containing six PAHs by OT-CEC with three different buffers: MES, Tris and phosphate. Experimental conditions: OTC, 41 cm (31 cm to the detector)× 9.60 μm I.D.; mobile phase, MeCN–1 mM buffer pH 7.0 (50:50); hydrodynamic injection, 15 cm for 15 s; applied voltage, 25 kV; detection, UV at 235 nm. The test mixture contained (1) DMF, (2) naphthalene, (3) acenaphthene, (4) phenanthrene, (5) anthracene, (6) fluoranthene and (7) pyrene.

is used at a pH near its pK_a (high buffering capability), a decrease in the EOF was observed going from high to low pH (the EOF is known to be dependent on the pH of the mobile phase due to its effect on the extent of dissociation of surface silanol groups [23]). However, only a significant effect on the EOF, analysis time, and efficiency was obtained changing the pH from 6 to 7 (see Table 2). Thus, the use of phosphate buffer at pH 7 was recommended because it is less dangerous than pH 8 for silica based stationary phases, and in addition a smaller baseline noise was obtained with this buffer (see Fig. 2).

3.3. Effect of the percentage of organic modifier in the mobile phase on the electroosmotic mobility, solute retention, and efficiency

Following the selection of the concentration and nature of the buffer, the effect of the MeCN percentage was evaluated in the range 50–65% v/v (50% was the minimum required for solubilization of analytes, and 65% was the maximum without loss of the baseline resolution in the separation of the compounds in the test mixture). When hydro–organic mixtures are used, the ε_r/η ratio of the solvent will change with the composition of the mobile phase (see Fig. 3) and therefore, a change in u_{eo} could also be expected according to Eq. (1).

The effect of MeCN percentage in the mobile phase, while maintaining all other separation parameters constant, is shown in Table 3. An increase in the percentage of organic modifier in the mobile phase over the range 50–65% resulted in an increase in the electroosmotic mobility. This result was also observed in packed-CEC with columns of C_{18}-silica gel [24,25], but it is in contradiction with the result

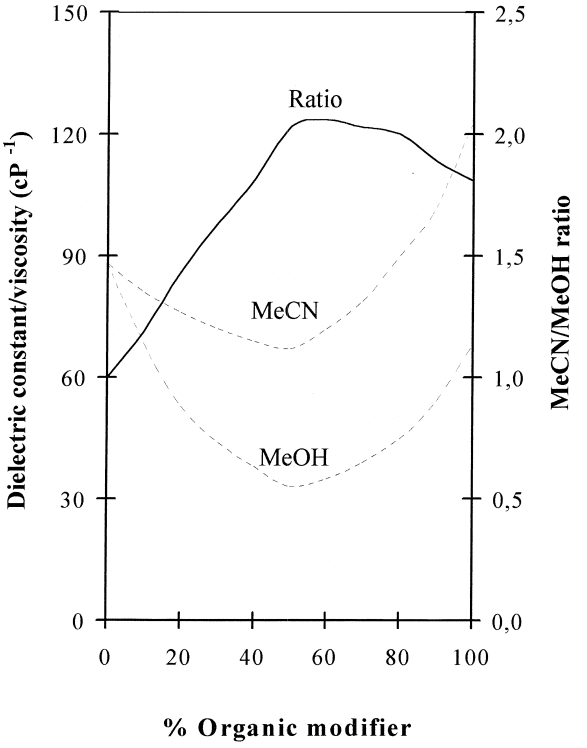

Fig. 3. Plot of the ratio dielectric constant (ε_r) over viscosity (η) for MeOH–water and MeCN–water (dashed lines). The solid line represents the ratio between both. Data from Schwer and Kenndler [11].

Table 2
Effect of type and pH of buffer used in OT-CEC. Values are the average of at least three replicates (RSD$_{\mu_{eo}}$ = 0.6–1.6%; RSD$_k$ = 1.7–4.7%; RSD$_H$ = 1.8–4.1%; RSD$_t$ = 0.6–1.6%). Experimental conditions as stated in Fig. 2

Buffer	μ_{eo} ($\times 10^{-4}$ cm^2/V s)	Pyrene		
		k	H (μm)	t_r (min)
Tris (pH 8.0)	2.40	0.42	3.0	4.2
Tris (pH 7.0)	2.39	0.37	3.1	4.4
Phosphate (pH 7.0)	2.37	0.43	3.2	4.6
MES (pH 7.0)	2.34	0.41	3.1	4.4
MES (pH 6.0)	1.52	0.39	4.3	6.9

Table 3
Effect of MeCN concentration in the mobile phase on the electroosmotic mobility (μ_{eo}), the linear velocity (u_{eo}; RSD$_{n=3}$ = 0.5–1.2%), the retention factor (k; RSD$_{n=3}$ = 0.6–3.0%), the plate height (H; RSD$_{n=3}$ = 0.6–5.1%) and the electron time (t_r; RSD$_{n=3}$ = 0.5–1.2%). Experimental conditions: OTC, 41 cm (31 cm to the detector) × 9.60 μm I.D.; mobile phase, MeCN–1 m*M* phosphate buffer pH 7.0; hydrodynamic injection, 15 cm for 15 s; applied voltage, 22.5 kV; detection, UV at 220 nm

MeCN (%)	μ_{eo} ($\times 10^{-4}$ cm^2/V s)	u_{eo} (cm/s)	Pyrene		
			k	H (μm)	t_r (min)
65	3.18	0.176	0.12	2.2	3.3
60	3.04	0.168	0.17	2.3	3.6
55	2.80	0.155	0.27	2.7	4.2
50	2.40	0.133	0.44	3.2	5.6

obtained for bare fused-silica capillaries [11]. Therefore, this result could not be explained only by an increase on ε_r/η ratio of the mobile phase (see Fig. 3), also an increase in the silanol density on the surface of the layer of silica gel in the capillary wall could contribute to EOF [26]. Thus, the polarity decrease of the mobile phase due to the increase of MeCN percentage could make more effective the drenching of the C_{18} moieties of the stationary phase, which would originate a larger density of bare free silanol groups of silica gel layer on the capillary wall.

On the other hand, solutes retention increased significantly when the MeCN content in the mobile phase was decreased. Thus, the natural logarithm of the retention factor of the neutral compounds in the test mixture exhibited a good linear relationship with the percentage of MeCN in the mobile phase ($y = -0.087x + 2.033$ with $r^2 = 0.999$ for naphthalene, $y = -0.090\ x + 3.090$ with $r^2 = 0.999$ for phenanthrene and $y = -0.090x + 3.644$ with $r^2 = 0.998$ for pyrene, number of points = 12). In addition, a similar slope was obtained for all compounds studied, which justified the use of DMF as a measure of the EOF. This linear relationship is a clear example of the reversed-phase (RP) like selectivity of the OTC-C_{18} used. This result suggested that the separation of neutral solutes by CEC can be achieved with partitioning as the main retention mechanism. Therefore, in principle, the well-established theories to develop RP-HPLC methods should be equally applicable to the separation of neutral species by CEC with one exception: if optimization of a separation by variation of the mobile phase composition is desired, the dependence of the EOF on the mobile phase composition must be taken into account.

Finally, the plate height ranged from 2.2 to 3.2 μm and it was observed that an increase in the content of MeCN in the mobile phase generated greater efficiencies, i.e. smaller plate height, mainly due to the decrease in the retention. The plate heights presented in this work are better than the described for the same OTCs in HPLC [3], plate height of 5.6 μm for a k value of 0.24, and corroborate the described in theory studies according to which the flow profile in CEC is much flatter than the parabolic flow profile of HPLC increasing the efficiency of an OTC by a factor larger than 2 [6].

3.4. Effect of the type of organic modifier in the mobile phase on the electrochromatographic separation of neutral solutes

The same behaviour described before was ob-

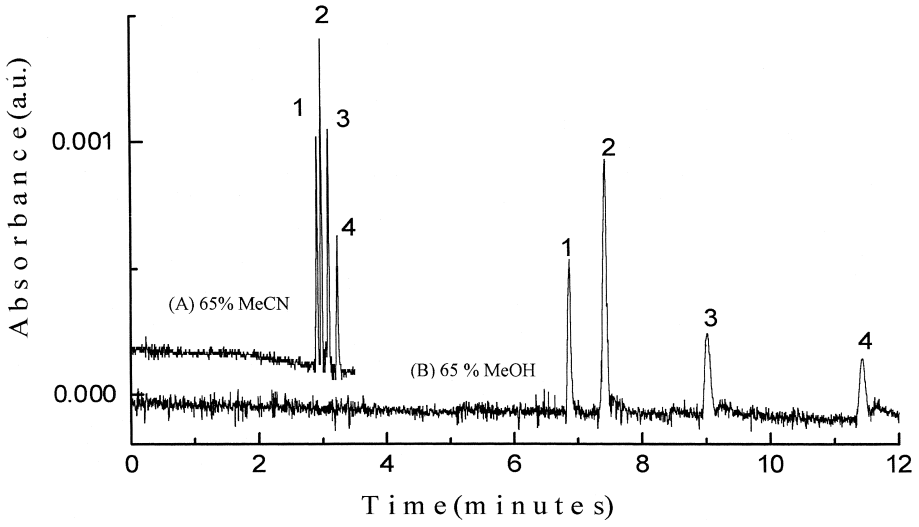

Fig. 4. Separation demonstrating the resolving power of MeOH. Experimental conditions: OTC, 41 cm (31 cm to the detector)×9.60 μm I.D.; mobile phase, 65% (A) MeCN or (B) MeOH and 35% 1 m*M* phosphate buffer (pH 7.0); hydrodynamic injection, 15 cm for 15 s; applied voltage, 22.5 kV; detection, UV at 220 nm. Test compounds were (1) DMF, (2) naphthalene, (3) phenanthrene and (4) pyrene.

served when using MeOH as organic modifier (data not shown). However, MeCN is known to have a higher elution strength than MeOH and according to Eq. (1) the magnitude of the mobile phase velocity varies with the type of organic modifier being for MeCN higher than for MeOH due to the ε_r/η ratio (see Fig. 3). Thus, the choice of the organic modifier (MeCN or MeOH) is critical because it affects the separation selectivity and the level of EOF, and, therefore, the analysis time (see Fig. 4).

Fig. 5 illustrates the separation of the eight PAHs studied under optimal conditions using a mobile phase 1 m*M* in phosphate buffer (pH 7.0) containing 50% MeCN and an applied voltage of 30 kV. Efficiencies measured for naphthalene and anthracene were 115 000 plates with a *k* value of 0.10 for the first and 106 000 plates with a k value of 0.30 for the second. These values correspond to plate heights of 2.6 μm and 2.8 μm, respectively, at a linear velocity of 0.19 cm/s and an analysis time of only 4 min. Fig. 6 shows the separation of the same PAHs using a mobile phase containing 65% MeOH and the other conditions as in Fig. 5. In this electrochromatogram, plate numbers of 89 000 and 70 000 for naphthalene ($k=0.11$) and anthracene ($k=0.44$), respectively, were measured, which correspond to plate heights of 3.4 μm and 4.3 μm, respectively, at a linear velocity of 0.09 cm/s. A comparison of the electrochromatograms of Figs. 5 and 6 indicates that

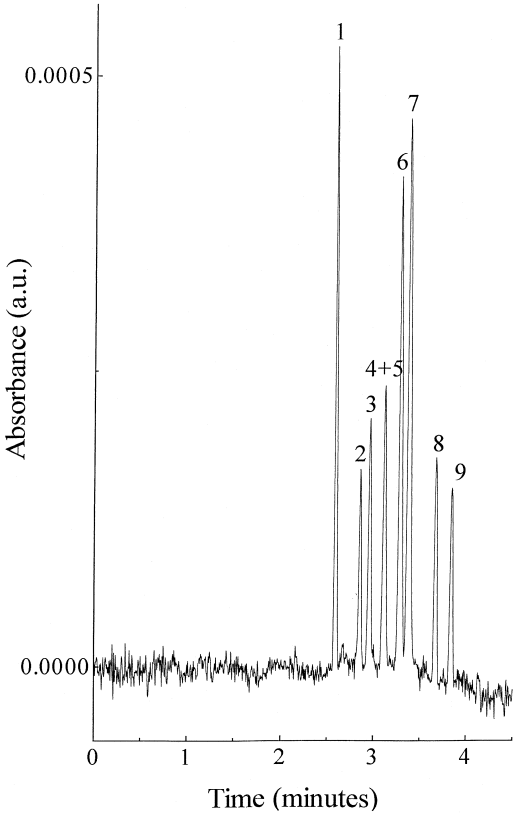

Fig. 5. Electrochromatogram corresponding to a test mixture containing eight PAHs when a mixture of MeCN–1 m*M* phosphate buffer pH 7.0 (50:50) was used. Experimental conditions: OTC, 40 cm (30 cm to the detector) 9.60 μm I.D.; hydrodynamic injection, 15 cm for 15 s; applied voltage, 30 kV; detection, UV at 235 nm. Test compounds were (1) DMF, (2) naphthalene ($k=0.10$), (3) acenaphthylene ($k=0.14$), (4) acenaphthene ($k=0.20$), (5) fluorene ($k=0.20$), (6) phenanthrene ($k=0.27$), (7) anthracene ($k=0.30$), (8) fluoranthene ($k=0.42$) and (9) pyrene ($k=0.48$).

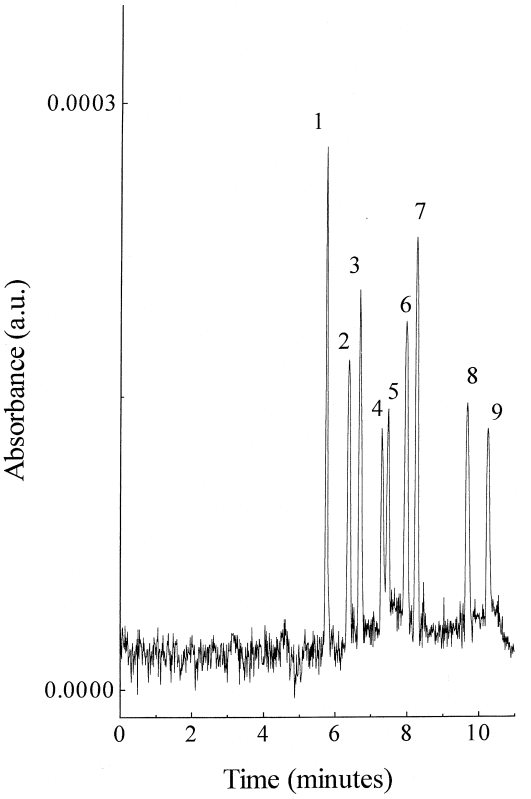

Fig. 6. Electrochromatogram of eight PAHs separated with a mixture of MeOH–1 m*M* phosphate buffer pH 7.0 (65:35). Other experimental conditions as in Fig. 5. Test compounds were (1) DMF, (2) naphthalene ($k=0.11$), (3) acenaphthylene ($k=0.16$), (4) acenaphthene ($k=0.27$), (5) fluorene ($k=0.30$), (6) phenanthrene ($k=0.39$), (7) anthracene ($k=0.44$), (8) fluoranthene ($k=0.68$) and (9) pyrene ($k=0.78$).

faster analysis can be reached in CEC when MeCN instead of MeOH was used as organic modifier due to the increase in the EOF velocity. In addition, the use of MeCN permits to obtain better efficiencies than MeOH, which could be explained by both the larger flow velocity and the smaller viscosity of MeCN. On the other hand, the use of MeOH or MeCN in the mobile phase enables to obtain different selectivity as in HPLC. Thus, the fact that the selectivity between acenaphthylene and fluorene is 1.9 with MeOH and 1.4 with MeCN makes possible the separation of acenaphthene and fluorene when MeOH is used in the mobile phase instead of MeCN.

4. Conclusions

The potential of OT-CEC looks promising. The linear velocities have been found to be adequate when typical reversed-phase mobile phases are used. However, the knowledge of the influence of the mobile phase composition in CEC is essential to achieve further enhancement of separation performance. Thus, the use of a low buffer concentration (1 m*M*) provided the highest resolution with the smallest separation time. There are no significant differences on the separations of organic compounds such PAHs when buffers at the same pH but different nature, inorganic (phosphate) or organic (Tris or MES), were used. However, a decrease in the EOF was observed when a buffer of pH 6 (i.e., MES) was used instead of buffers of pH 7 or 8 (i.e., phosphate or Tris, respectively).

On the other hand, as in HPLC, the type of organic modifier used affects separation selectivity in CEC. In addition, the magnitude of the EOF varies with the type and concentration of the organic modifier. The use of MeCN could be advantageous to increase the separation speed while MeOH enables to obtain larger retention factors and different separation selectivity.

Separation of neutral solutes by OT-CEC can be achieved by means of typical reversed-phase HPLC conditions, being partitioning the main retention mechanism. Therefore, in principle, the transfer of HPLC methods to CEC, and the exploitation of the higher efficiency to improve the method should be feasible.

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