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Parameters controlling the elution window and retention factors in micellar electrokinetic capillary chromatography

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Abstract

The resolution of uncharged compounds in micellar electrokinetic capillary chromatography (MECC) is influenced by both the elution window and the retention factors of the compounds. The influence of the electroosmotic mobility and the effective mobility of the micelles on the elution window is treated theoretically and the effect of different experimental conditions on the elution window and the retention factors is determined. Although these variables cannot be controlled independently in many cases, the resolution in MECC can be improved by adjusting the composition of the applied electrolyte system. This is demonstrated for several electrolyte systems with different pH values, ionic strengths, surfactant concentrations and organic modifier contents. Further, the influence of the applied field strength, capillary surface modifications and the alkyl chain length of the surfactant are evaluated.

1. Introduction

Micellar electrokinetic capillary chromatography (MECC), has proved to be a highly efficient separation method for the determination of neutral compounds. Applying the same instrumentation as in capillary zone electrophoresis, in MECC uncharged compounds can be separated based on differences in their partitioning between two phases, just as in chromatographic techniques. Since the introduction of MECC by Terabe et al. [1,2], several authors have paid attention to the fundamental characteristics of this separation method [3,4] and to the effect of different separation parameters on the migration behavior [5–11]. Also the theoret-

ical [12,13] and practical [14,15] aspects of resolution optimization have been well described.

1.1. Elution window and migration modes

The separation mechanism of neutral compounds in MECC is based on their partitioning between two moving phases, viz. an electroosmotically pumped aqueous mobile phase and a pseudostationary micellar phase. The distribution between these two phases is expressed by the retention factor, k , which can be calculated according to [1,2]:

$$k = \frac{\eta_{MC}}{\eta_{AO}} = \frac{t_S - t_{EOF}}{t_{EOF} \left(1 - \frac{t_S}{t_{MC}}\right)} \quad (1)$$

where η_{MC} and η_{AO} are the numbers of moles of

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the solute in the micellar and the aqueous phase, and t_s , t_{EOF} and t_{MC} are the migration times of the solute, the electroosmotic flow (EOF) and the micelles, respectively. All neutral compounds will migrate with an overall linear velocity, v_s , according to:

$$v_s = \frac{1 + \frac{t_{\text{EOF}}}{t_{\text{MC}}} k}{1 + k} m_{\text{EOF}} E \quad (2)$$

where m_{EOF} and E are the electroosmotic mobility and the applied field strength, respectively. They will be detected within the time interval between t_{EOF} and t_{MC} [2], which is called the elution window. Under conditions of a constant field strength, the elution window is determined by the electroosmotic mobility, m_{EOF} , and the effective mobility of the micelles, m_{MC} , according to:

$$\frac{t_{\text{MC}}}{t_{\text{EOF}}} = \frac{m_{\text{EOF}}}{m_{\text{EOF}} + m_{\text{MC}}} \quad (3)$$

The elution window, i.e. $t_{\text{MC}}/t_{\text{EOF}}$, can be increased by increasing $|m_{\text{MC}}|$ or by decreasing m_{EOF} . If the absolute value of the effective mobility of the micelles exceeds the electroosmotic mobility, the elution window becomes negative.

As described by Vindevogel and Sandra [4], three different migration modes can be distinguished. If $t_{\text{MC}}/t_{\text{EOF}} > 0$, all compounds will migrate to the detector, which is called the normal mode. If $t_{\text{MC}}/t_{\text{EOF}} < 0$, only compounds for which holds $k < -(t_{\text{MC}}/t_{\text{EOF}})$ will migrate to the detector. This mode is called the restricted elution mode. Compounds for which holds $k > -(t_{\text{MC}}/t_{\text{EOF}})$ will migrate in the opposite direction. In order to detect these compounds a reversed polarity will have to be applied, which is called the reversed direction mode.

1.2. Resolution equation

The basic resolution equation for the resolution, R_s , between two closely eluting peaks (assuming $k \approx k_1 \approx k_2$) in MECC is given by [2,4]:

$$R_s = \frac{\sqrt{N}}{4} \frac{\alpha - 1}{\alpha} \frac{k}{1 + k} \frac{1 - \frac{t_{\text{EOF}}}{t_{\text{MC}}}}{1 + \frac{t_{\text{EOF}}}{t_{\text{MC}}} k} \quad (4)$$

where N and α are the number of theoretical plates and the separation factor k_2/k_1 , respectively. The last term in Eqn. (5) reflects the specific resolution characteristics in MECC. The influence of the retention factor on the resolution is described by the retention term $f(k)$, formed by the product of the last two terms in Eqn. (4):

$$f(k) = \frac{k}{1 + k} \frac{1 - \frac{t_{\text{EOF}}}{t_{\text{MC}}}}{1 + \frac{t_{\text{EOF}}}{t_{\text{MC}}} k} \quad (5)$$

Graphs of $f(k)$ versus k in the normal mode show that increasing the elution window with a given retention factor leads to a higher value of $f(k)$ [2]. For each value of the elution window, the optimum retention factor, k_{opt} , can be calculated by differentiating Eqn. (5) with respect to k [12,16]:

$$k_{\text{opt}} = \sqrt{t_{\text{MC}}/t_{\text{EOF}}} \quad (6)$$

In the restricted elution mode and in the reversed direction mode the contribution of $f(k)$ to the resolution can reach much higher values than in the normal mode. However, this will lead to long analysis times, especially for compounds with a retention factor close to $-(t_{\text{MC}}/t_{\text{EOF}})$ [6].

Thus, assuming a constant efficiency and the selectivity being determined by the nature of the surfactant system, the resolution is mainly influenced by the elution window and the retention factors of the sample compounds. Therefore a good understanding of the effects of different experimental conditions on both these variables is important for the development of MECC analyses and for resolution optimization strategies. In this work, we studied the influence of the applied field strength, buffer pH, ionic strength, capillary surface modifications, alkyl chain length of the surfactant, surfactant concentration and organic modifier content on the elution window

and the retention factors of uncharged compounds in MECC.

2. Experimental

2.1. Instrumentation and separation conditions

All experiments were carried out on a BioFocus 3000 Capillary Electrophoresis System (BioRad, Hercules, CA, USA) at a constant temperature of 25°C. The wavelength of the detector was set at 200 nm. Pressure injection was carried out with an injection constant of 2 psi.s. All experiments were carried out with a constant voltage, with the anode placed at the inlet side and the cathode at the outlet side of the capillary, respectively. Two different fused-silica capillaries of 50 μm I.D. were applied; an original BioRad standard capillary, total length 50.0 cm, distance between injection and detection 45.5 cm, and a capillary from Supelco (Bellefonte, PA, USA), total length 70.0 cm, distance between injection and detection 65.4 cm. For some of the experiments several different coated fused-silica capillaries of 50 μm I.D. were applied; a C_{18} coated capillary from Supelco (CElect-H250, Bellefonte, PA, USA), a methyl silicone coated capillary and a polyethylene glycol coated capillary, both from Chrompack (Middelburg, The Netherlands), all with total length 70.0 cm, distance between injection and detection 65.4 cm. All experiments with the BioRad standard capillary were carried out three times, except for the experiments with the organic modifiers, which were carried out two times. The reported values are the average values.

2.2. Samples and solutions

All chemicals were of analytical-reagent grade. In Table 1 the compositions of the background electrolytes at different pH values are listed. To these buffer solutions 50 mM sodium dodecyl sulphate (SDS) was added, unless otherwise noted. Eight aromatic compounds were selected as sample compounds, covering a wide range of

Table 1
Composition of background electrolytes at different pHs

Cation ^a (0.01 M)	Buffering counter species ^a	pH
TRIS	Acetic acid	4.9
TRIS	MES	6.2
TRIS	o-Phosphoric acid	7.0
TEA	MOPS	7.0
TEA	HEPES	7.5
TRIS	MOPS	7.9
TRIS	MOPS	8.2
TRIS	Acetic acid	8.2
TRIS	Boric acid	8.5

^a TRIS = tris(hydroxymethyl)aminomethane; TEA = triethanolamine; MES = 2-(N-morpholino)ethanesulphonic acid; MOPS = morpholinopropanesulphonic acid; HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

hydrophobicity. All sample compounds were dissolved at a final concentration of about 0.0005 M in a 50 mM SDS solution. Formamide was used as a neutral EOF marker and Sudan III as a micelle marker to measure t_{EOF} and t_{MC} , respectively.

3. Results and discussion

3.1. Applied field strength

According to Eqn. (2) the velocity of the sample compounds is linearly related to the applied field strength. Although a linear relationship was obtained at low field strengths, a positive deviation was observed at field strengths above ca. 200 V/cm. At a higher field strength the generated electric power will increase. Due to Joule heating, the mean temperature in the capillary will increase, which in turn will result in a decrease of the viscosity of the electrolyte solution. Both the electroosmotic mobility and the effective mobility of the micelles are inversely proportional to the viscosity.

The specific conductivity of the electrolyte solution, κ , can be calculated by Ohm's law:

$$V = \frac{1}{\pi r^2 \kappa} I \quad (7)$$

where V is the applied voltage, l and r are the length and the radius of the capillary, respectively, and I is the measured electric current. Since the specific conductivity is inversely proportional to the viscosity, the electric current will proportionally increase if the viscosity decreases at a higher field strength. Therefore, a linear relationship was obtained for field strengths up to 400 V/cm between the velocity of the sample compounds and the measured electric current with regression correlation coefficients larger than 0.999. In Table 2 the measured electric current, the calculated specific conductivity, the electroosmotic mobility, the effective mobility of the micelles and the corresponding values for the elution window are listed for the different applied voltages.

3.2. Buffer pH and ionic strength

In fused-silica capillaries, the EOF originates from the dissociation of the surface silanol groups of the capillary wall. Therefore the electroosmotic mobility is dependent on the pH of the background electrolyte and, according to the double layer theory, also on the ionic strength. In Fig. 1 the pH dependence of the EOF is illustrated for phosphate buffers. As expected, a sigmoidal curve was obtained for m_{EOF} versus pH, also for the phosphate buffers containing 50 mM SDS. The lower m_{EOF} obtained with the buffers containing 50 mM SDS at high and intermediate pHs is due to an increase in viscosity and ionic strength of the electrolyte systems.

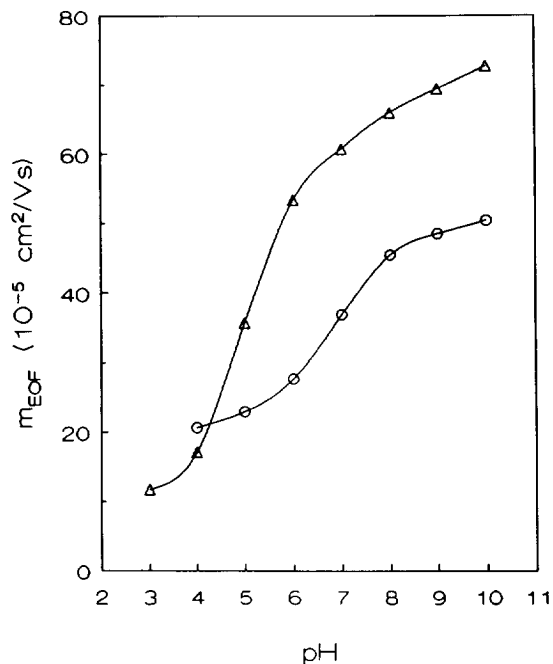


Fig. 1. Dependence of the electroosmotic mobility, m_{EOF} , on the pH for phosphate buffers, containing (Δ) 0 and (\circ) 50 mM SDS. The phosphate buffers were prepared by adding *o*-phosphoric acid to a 10 mM KOH solution until the desired pH was reached. Capillary from Chrompack. Applied voltage, 20 kV.

The more slightly decrease observed at lower pHs is due to adsorption of SDS on the inner wall of the capillary. For a given surfactant, the effective mobility of the micelles can be expected to be almost independent of pH [6]. In order to examine the influence of buffer pH and ionic

Table 2

Applied voltage, V (kV), measured electric current, I (μA), specific conductivity, κ (S/m), electroosmotic mobility, m_{EOF} (10^{-5} cm^2/Vs), effective mobility of the micelles, m_{MC} (10^{-5} cm^2/Vs), and values for the elution window, $t_{\text{MC}}/t_{\text{EOF}}$, for different applied field strengths. BioRad standard capillary. Background electrolyte, 10 mM TRIS-acetic acid at pH 8.2.

V	I	κ	m_{EOF}	m_{MC}	$t_{\text{MC}}/t_{\text{EOF}}$
3	3.0	0.255	61.96	-40.71	2.92
6	6.0	0.255	62.20	-40.54	2.87
9	9.2	0.260	63.48	-41.21	2.85
12	12.7	0.270	65.69	-42.47	2.83
15	16.7	0.284	68.50	-44.21	2.82
18	21.3	0.301	71.25	-45.88	2.81
20	24.3	0.309	73.39	-47.18	2.80

strength on the elution window and the retention factors in MECC, experiments were carried out with the electrolyte systems given in Table 1, containing 50 mM SDS. In addition, experiments were carried out with electrolyte systems containing 0.02 M of the cation and 100 mM SDS. In Table 3 all calculated electroosmotic mobilities, effective mobilities of the micelles, corresponding values of the elution window and retention factors of the eight sample compounds are listed. As can be seen from these results the electroosmotic mobility is dependent on pH and ionic strength, whereas the effective mobility of the micelles and the retention factors of the

compounds are virtually constant for a given surfactant concentration. Of course this only applies for uncharged compounds in MECC. For charged compounds, the degree of dissociation and hence the retention factor may be strongly influenced by the pH of the electrolyte system [17]. The retention factors obtained with 100 mM SDS are less than twice those obtained with 50 mM SDS for all compounds, which is inconsistent with theory. Due to a higher electric current with the 100 mM SDS electrolyte system, the mean temperature in the capillary will increase. This will cause a decrease of distribution constants and retention factors of the sample

Table 3
Electroosmotic mobility, m_{EOF} ($10^{-5} \text{ cm}^2/\text{Vs}$), effective mobility of the micelles, m_{MC} ($10^{-5} \text{ cm}^2/\text{Vs}$), values for the elution window, $t_{\text{MC}}/t_{\text{EOF}}$, and retention factors, k , for (1) resorcinol, (2) phenol, (3) *p*-nitroaniline, (4) *p*-cresol, (5) 2,6-xyleneol, (6) toluene, (7) 1,2-xylool and (8) propylbenzene, in two background electrolytes at different pHs. BioRad standard capillary. Applied voltage, 15 kV

pH	m_{EOF}	m_{MC}	$t_{\text{MC}}/t_{\text{EOF}}$	k							
				1	2	3	4	5	6	7	8
<i>Cation concentration 10 mM, containing 50 mM SDS</i>											
6.2	58.07	-44.31	4.22	0.22	0.52	1.14	1.47	2.87	3.33	8.24	24.79
7.0 ^a	56.43	-43.97	4.53	0.25	0.56	1.22	1.55	2.82	3.22	7.94	24.27
7.5	61.26	-44.34	3.62	0.25	0.56	1.23	1.56	2.82	3.20	7.84	23.67
7.9	64.59	-44.68	3.24	0.27	0.56	1.19	1.51	2.60	3.10	7.66	23.32
8.2 ^b	66.46	-44.95	3.09	0.28	0.56	1.19	1.51	2.74	3.12	7.62	23.06
8.2 ^c	65.94	-44.00	3.00	0.24	0.51	1.11	1.42	2.63	3.02	7.46	22.48
8.5	66.75	-44.18	2.96	0.27	0.53	1.14	1.45	2.68	3.23	7.49	22.92
Average		-44.35		0.25	0.54	1.17	1.50	2.74	3.17	7.75	23.50
Standard deviation		0.33		0.02	0.02	0.04	0.05	0.10	0.09	0.26	0.75
<i>Cation concentration 20 mM, containing 100 mM SDS</i>											
7.5	52.88	-44.29	6.02	0.36	0.95	1.90	2.61	4.70	5.92	14.29	43.40
7.9	56.42	-44.45	4.72	0.35	0.91	1.81	2.49	4.54	5.76	13.95	42.40
8.2 ^d	57.98	-44.32	4.24	0.37	0.93	1.87	2.56	4.64	5.83	14.02	42.83
8.2 ^e	59.90	-45.09	4.05	0.38	0.93	1.86	2.55	4.66	5.84	14.20	43.02
8.5	60.14	-44.90	3.95	0.41	0.97	1.94	2.65	4.83	6.01	14.48	43.99
Average		-44.61		0.37	0.94	1.88	2.57	4.67	5.87	14.20	43.13
Standard deviation		0.32		0.02	0.02	0.04	0.05	0.09	0.09	0.18	0.54

^a 0.01 M TEA-MOPS.

^b 0.01 M TRIS-MOPS.

^c 0.01 M TRIS-acetic acid.

^d 0.02 M TRIS-MOPS.

^e 0.02 M TRIS-acetic acid.

compounds [18,19]. In Fig. 2 the relationship between t_{MC}/t_{EOF} and m_{EOF} for the experiments, listed in Table 3, is illustrated.

From the foregoing it can be concluded that for a given separation of neutral species with a specific surfactant, the pH of the electrolyte system can be used to optimize the elution window in MECC. This is demonstrated in Fig. 3, where the electrokinetic chromatograms are shown for the separation of the sample mixture in an electrolyte system of 0.01 M TRIS–boric acid at pH 8.5 and 0.01 M TRIS–acetic acid at pH 4.9, respectively. In order to determine the effective mobility of the micelles and to be able to calculate retention factors with the low m_{EOF} at pH 4.9, Sudan III was injected at the outlet side of the capillary by electrokinetic injection with 10 kV for 10 s, after the hydrodynamic

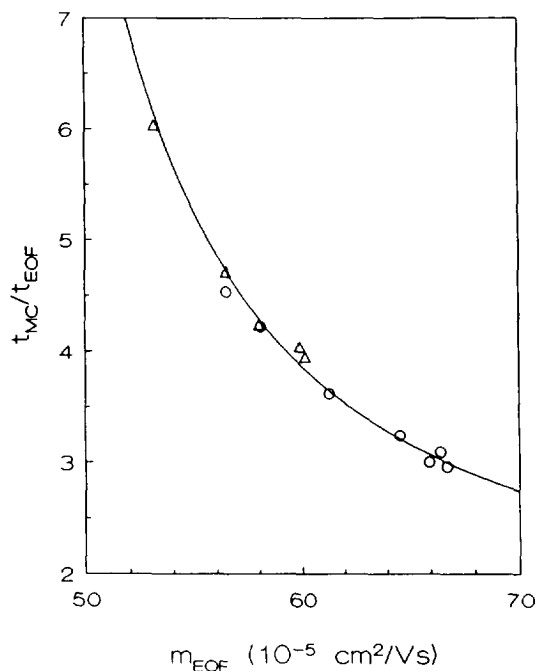


Fig. 2. Relationship between elution window and electroosmotic mobility for the electrolyte systems at different pHs with (○) a cation concentration of 0.01 M, containing 50 mM SDS and (△) a cation concentration of 0.02 M, containing 100 mM SDS. The drawn line represents the theoretical curve for an m_{MC} of $-44.46 \cdot 10^{-5} \text{ cm}^2/\text{Vs}$. BioRad standard capillary. Applied voltage, 15 kV.

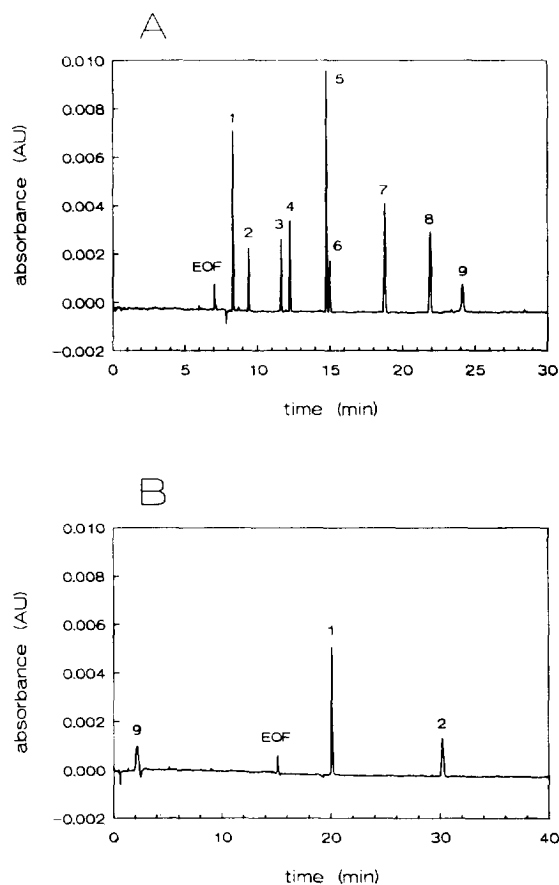


Fig. 3. Electrokinetic chromatograms of the separation of (1) resorcinol, (2) phenol, (3) *p*-nitroaniline, (4) *p*-cresol, (5) 2,6-xyleneol, (6) toluene, (7) 1,2-xylool, (8) propylbenzene and (9) Sudan III in a background electrolyte of (A) 10 mM TRIS–boric acid at pH 8.5 and (B) 10 mM TRIS–acetic acid at pH 4.9. Capillary from Supelco. Applied voltage, 20 kV. For further explanation, see text.

injection of the sample mixture at the inlet side of the capillary. In Table 4 all measured migration times and calculated retention factors are listed. From these results it can be calculated that a change in t_{MC}/t_{EOF} is obtained from 3.43 to -2.08 if the pH of the electrolyte system is lowered from 8.5 to 4.9. Consequently a higher resolution is obtained, however, at the cost of a longer analysis time. Notice that in Fig. 3A all compounds are migrating in the normal mode. In Fig. 3B resorcinol and phenol are migrating in

Table 4
Migration times, t (min), and retention factors, k , with standard deviations (in parentheses), and theoretical plate numbers, N , in two background electrolytes. Capillary from Supelco. Applied voltage, 20 kV. ($n = 5$)

Compound	t	k	$N \cdot 10^{-5}$
<i>10 mM TRIS–boric acid at pH 8.5</i>			
EOF	7.01 (0.04)	0	–
Resorcinol	8.26 (0.05)	0.272 (0.001)	1.35
Phenol	9.35 (0.03)	0.547 (0.005)	1.52
<i>p</i> -Nitroaniline	11.64 (0.03)	1.283 (0.024)	1.69
<i>p</i> -Cresol	12.27 (0.02)	1.538 (0.029)	1.87
2,6-Xylenol	14.78 (0.02)	2.889 (0.053)	2.24
Toluene	15.03 (0.02)	3.061 (0.059)	2.32
1,2-Xylol	18.77 (0.03)	7.702 (0.142)	2.24
Propylbenzene	21.82 (0.07)	23.172 (0.449)	1.75
Sudan III	24.01 (0.11)	∞	1.73
<i>10 mM TRIS–acetic acid at pH 4.9</i>			
EOF	15.11 (0.07)	0	–
Resorcinol	19.98 (0.13)	0.197 (0.001)	1.64
Phenol	29.77 (0.31)	0.499 (0.007)	1.31
Sudan III ^a	2.21 (0.04)	∞	–

^a Note that the length from injection to detection is only 4.6 cm for Sudan III. For further explanation, see text.

the restricted elution mode, whereas Sudan III is migrating in the reversed direction mode.

3.3. Capillary surface modifications

Besides the composition of the electrolyte system, the electroosmotic mobility can be controlled by modification of the capillary surface [20–22]. To investigate the influence of several surface modifications in MECC, the sample mixture was analysed with one uncoated and three different coated fused-silica capillaries in a 0.01 M TRIS–phosphoric acid electrolyte system at pH 7.0. In Fig. 4 all electrokinetic chromatograms are shown. The C₁₈ and the polyethylene glycol coated capillaries showed a decrease in m_{EOF} , resulting in a larger elution window. However, also a decrease in efficiency was observed, probably due to solute–wall interactions. The methyl silicone coated capillary showed an increase in m_{EOF} , resulting in a smaller elution window. Toluene, 1,2-xylol, propylbenzene and Sudan III could not be detected, owing to adsorption of these compounds on the polymer

coating. These results suggest that only for a limited number of compounds the application of coated capillaries in MECC may be advantageous.

3.4. Alkyl chain length of the surfactant

For micelles, the effective mobility will be strongly dependent on the nature of the surfactant. A shorter alkyl chain will lead to a reduced aggregation number and consequently to a reduced effective charge, but also to a reduced micelle size [23]. Both these factors influence the m_{MC} in an opposite way. Moreover, the m_{EOF} is also influenced by the nature of the surfactant. At equal surfactant concentrations, the phase ratio will decrease if a surfactant with a shorter alkyl chain is applied, due to an increase in the critical micelle concentration and a decrease in the partial molar volume of the micelles (see Eqn. 9), resulting in a decrease of the retention factors. Therefore the elution window and the retention factors cannot independently being

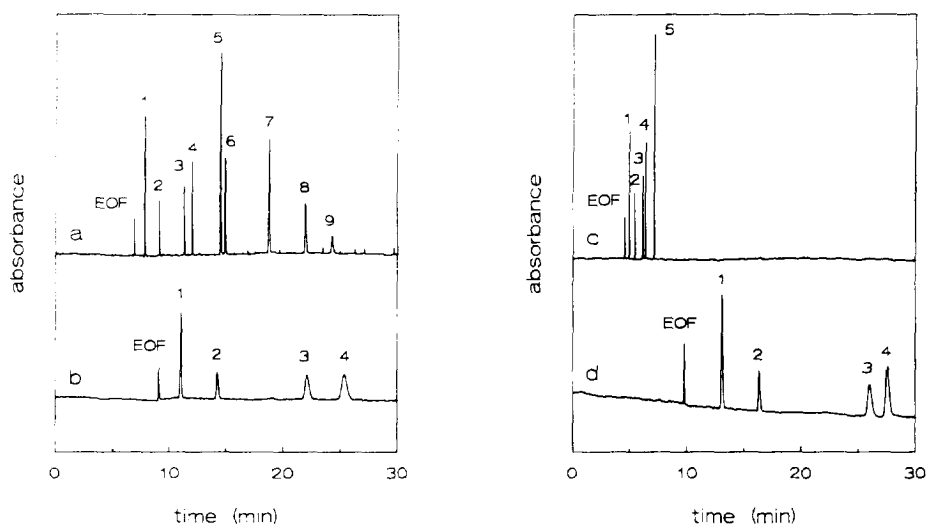


Fig. 4. Electrokinetic chromatograms of the sample mixture obtained with capillaries with different surface modifications: (a) untreated fused-silica capillary from Supelco, (b) C_{18} coated capillary from Supelco, (c) methyl silicone coated capillary from Chrompack and (d) polyethylene glycol coated capillary from Chrompack. Background electrolyte, 10 mM TRIS–phosphoric acid at pH 7.0. Applied voltage, 20 kV. See the legend of Fig. 3 for the names of the compounds.

optimized by changing the alkyl chain length of the surfactant.

3.5. Surfactant concentration

The retention factor is related to the distribution constant, K_c , and the phase ratio, β , according to:

$$K_c = k \beta \quad (8)$$

The phase ratio can be calculated according to:

$$\beta = \frac{V_{AQ}}{V_{MC}} = \frac{1 - \bar{v} (C_{SF} - CMC)}{\bar{v} (C_{SF} - CMC)} \quad (9)$$

where V_{AQ} and V_{MC} are the volume of the aqueous phase and the micellar phase, respectively, \bar{v} is the partial molar volume of the micelles, C_{SF} is the surfactant concentration and CMC is the critical micelle concentration. Under normal MECC conditions the numerator of Eqn. (9) is approximately equal to 1, leading to:

$$k = K_c \bar{v} (C_{SF} - CMC) \quad (10)$$

Thus the retention factor is linearly related to the surfactant concentration [2].

To investigate the influence of the phase ratio on the retention factors and the elution window, experiments were carried out with an electrolyte system of 0.01 M TRIS–MOPS at pH 8.2, containing different concentrations SDS, ranging from 30 mM to 100 mM. For the retention factors versus concentration SDS linear graphs were obtained with regression correlation coefficients larger than 0.997. As can be seen from the results, listed in Table 5, both m_{EOF} and $|m_{MC}|$ decrease with increasing surfactant concentration, due to changes in viscosity and ionic strength. The increase in viscosity will be partly compensated by Joule heating with a higher electric current (see also section 3.1., *Applied field strength*). Here it should be noted that in capillary electrophoretic techniques a distinction can be made between bulk viscosity (important for m_{MC}) and wall–surface viscosity (important for m_{EOF}) [24]. We assume that bulk viscosity will be more influenced by Joule heating than wall surface viscosity. As a result, a small increase of the elution window is observed at higher surfactant concentrations as also reported by others [4,25]. Terabe et al. [20], however, reported a small decrease of the elution window,

Table 5

Electroosmotic mobility, m_{EOF} (10^{-5} cm²/Vs), effective mobility of the micelles, m_{MC} (10^{-5} cm²/Vs), values for the elution window, $t_{\text{MC}}/t_{\text{EOF}}$, and measured electric current, I (μA), at different SDS concentrations, C_{SDS} (mM). BioRad standard capillary. Background electrolyte, 10 mM TRIS–MOPS at pH 8.2. Applied voltage, 15 kV

C_{SDS}	m_{EOF}	m_{MC}	$t_{\text{MC}}/t_{\text{EOF}}$	I
30	60.62	-43.18	3.47	11.1
40	59.48	-43.93	3.60	13.7
50	58.38	-42.53	3.69	16.7
60	58.29	-42.50	3.69	19.7
70	57.93	-42.34	3.72	22.7
80	57.49	-42.49	3.83	25.8
90	57.49	-42.76	3.91	29.6
100	57.15	-42.47	3.89	33.4

which may be due to differences in thermoregulating the capillary.

In Fig. 5 the effect of the surfactant concentration on the function $f(k)$ is demonstrated. For weakly hydrophobic compounds with $k < k_{\text{opt}}$ like resorcinol and phenol, an increase in $f(k)$ is observed with increasing surfactant con-

centration, whereas for strongly hydrophobic compounds with $k > k_{\text{opt}}$ like 1,2-xylyl a decrease in $f(k)$ is observed. For moderately hydrophobic compounds like *p*-cresol only a small influence of the surfactant concentration on $f(k)$ is observed.

3.6. Organic modifiers

Generally in MECC hydrophobic compounds show retention factors much larger than k_{opt} . The separation of these compounds can be improved by the addition of an organic modifier to the background electrolyte in order to decrease their retention factors to more favourable values. Moreover, the elution window will be extended by the addition of organic modifiers [10,11]. To study the influence of different organic modifiers in MECC, experiments were carried out with an electrolyte system of 0.01 M TRIS–boric acid at pH 8.5, containing different amounts of methanol, acetonitrile or urea. The migration times of the micelles for these experiments were calculated by an iteration procedure, applying the migration data of a homologous series of alkylbenzenes [19,26]. In Table 6 all calculated values for the electroosmotic mobility, the effective mobility of the micelles and the corresponding elution windows are summarized. At a methanol concentration above 20% (v/v) the restricted elution mode was obtained and t_{MC} could no longer be determined. A decrease in m_{EOF} and $|m_{\text{MC}}|$ is observed at increasing modi-

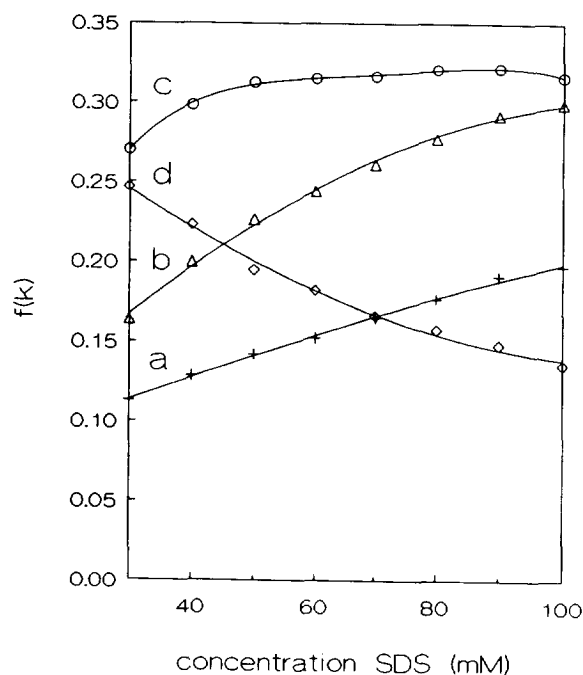


Fig. 5. Relationship between $f(k)$ and concentration SDS for (a) resorcinol, (b) phenol, (c) *p*-cresol and (d) 1,2-xylyl. BioRad standard capillary. Background electrolyte, 10 mM TRIS–MOPS at pH 8.2. Applied voltage, 15 kV.

Table 6

Electroosmotic mobility, m_{EOF} (10^{-5} cm²/Vs), effective mobility of the micelles, m_{MC} (10^{-5} cm²/Vs), and values for the elution window, $t_{\text{MC}}/t_{\text{EOF}}$, at different concentrations of methanol (%(v/v)), acetonitrile (%(v/v)) and urea (*M*). Background electrolyte, 10 mM TRIS–boric acid at pH 8.5. BioRad standard capillary. Applied voltage, 15 kV

Methanol				Acetonitrile				Urea			
%(v/v)	m_{EOF}	m_{MC}	$t_{\text{MC}}/t_{\text{EOF}}$	%(v/v)	m_{EOF}	m_{MC}	$t_{\text{MC}}/t_{\text{EOF}}$	<i>M</i>	m_{EOF}	m_{MC}	$t_{\text{MC}}/t_{\text{EOF}}$
0	67.14	–44.21	2.93	0	63.75	–44.11	3.25	0	58.40	–43.12	3.82
5	56.68	–39.18	3.24	5	58.86	–42.58	3.62	1	56.19	–41.99	3.96
10	48.90	–35.62	3.68	10	56.74	–42.41	3.96	2	54.97	–40.49	3.80
15	41.72	–31.96	4.28	15	52.78	–40.96	4.46	3	53.73	–41.03	4.23
20	36.64	–29.41	5.06	20	50.72	–42.08	5.87	4	52.88	–40.26	4.19
25	32.84	–	–	25	49.04	–41.47	6.48	5	51.43	–40.11	4.55
30	29.76	–	–	30	47.21	–41.45	8.22	6	50.71	–39.26	4.43
								7	49.04	–38.65	4.73

fier concentrations, due to changes in the viscosity and the dielectric constant of the electrolyte systems. Moreover, the micelle structure and hence m_{MC} will be influenced by the addition of an organic modifier. As a result, an increase in the elution window is observed with increasing concentrations of methanol, acetonitrile or urea.

In reversed-phase high-performance liquid chromatography it has been shown that the variation of the retention factor, *k*, with the volume fraction of organic solvent in the aqueous–organic mobile phase, ϕ , is reasonably well described by [27]:

$$\ln k = A + B\phi + C\phi^2 \quad (11)$$

where *A*, *B* and *C* are constants for a specific solute and eluent combination. For a small range of solvent compositions Eqn. (11) can be approximated by

$$\ln k = A + B\phi \quad (12)$$

Although in MECC small changes in the phase ratio may occur, due to the influence of organic modifiers on micelle structures, for methanol and urea a linear relationship was obtained between the logarithm of the retention factor and the concentration modifier in the background electrolyte, as shown in Fig. 6. For acetonitrile a 2nd order relationship was obtained. From Fig. 6 and Table 6 it can be concluded that with an increase

in modifier concentration a decrease in retention factors as well as an increase in elution window is obtained, resulting in a better resolution for hydrophobic compounds. As an example in Fig. 7 parts of the electrokinetic chromatograms are shown of the separation of alkylbenzenes with different amounts of methanol. As can be clearly seen a better resolution is obtained for strongly hydrophobic compounds migrating near t_{MC} at higher concentrations methanol, due to both a decrease in retention factor and an increase in elution window.

4. Conclusions

The overall linear velocity of neutral sample compounds was shown to be linearly related to the measured electric current. According to the resolution equation both the elution window and the retention factor influence the resolution in micellar electrokinetic capillary chromatography. These variables are often simultaneously affected by the experimental conditions and hence they cannot be controlled independently in many cases. The results demonstrate that the resolution of uncharged compounds can be improved by adjusting different separation parameters. The elution window can be increased by decreasing the pH of the electrolyte system, whereas the retention factors of the compounds remain fairly

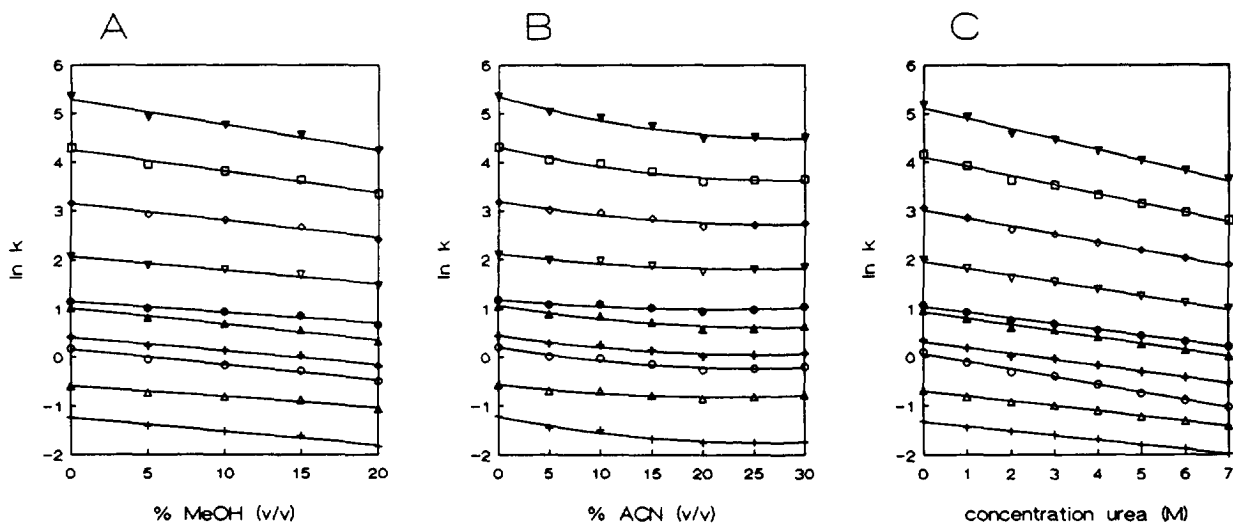


Fig. 6. Logarithm of retention factor versus concentration modifier for (A) methanol (% v/v), (B) acetonitrile (% v/v) and (C) urea (M). BioRad standard capillary. Background electrolyte, 10 mM TRIS–boric acid at pH 8.5, containing different amounts of organic modifier. Applied voltage, 15 kV.

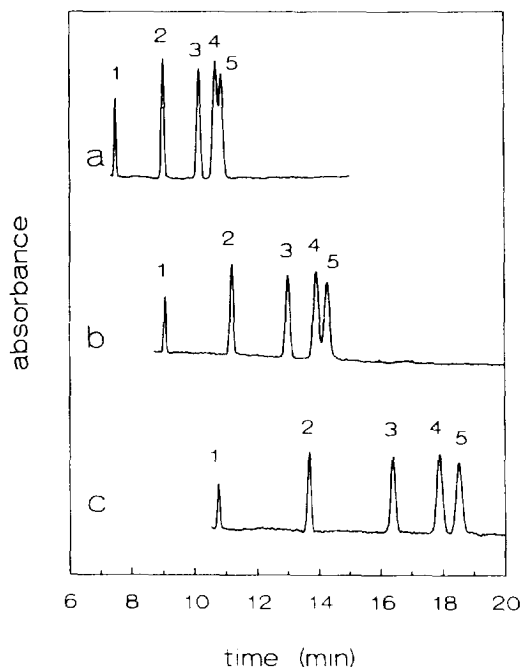


Fig. 7. Electrokinetic chromatograms of the separation of the alkylbenzenes (1) benzene, (2) toluene, (3) ethylbenzene, (4) propylbenzene and (5) butylbenzene in a background electrolyte of 10 mM TRIS–boric acid at pH 8.5, containing (a) 0%, (b) 5% and (c) 10% (v/v) methanol. BioRad standard capillary. Applied voltage, 15 kV.

constant. With a low electroosmotic flow at low pH values, the restricted elution mode can be obtained, resulting in a better resolution for weakly hydrophobic compounds. The use of coated capillaries was shown to be of limited value in MECC. The retention factor can be optimized in order to increase the function $f(k)$ by changing the surfactant concentration. An increase in elution window as well as a decrease in retention factors can be obtained by the addition of an organic modifier to the background electrolyte, resulting in a better resolution for strongly hydrophobic compounds. For methanol and urea a linear relationship is obtained between the logarithm of the retention factor and the modifier concentration whereas for acetonitrile a 2nd order relationship is obtained.

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