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Widening of the elution window in micellar electrokinetic chromatography with cationic surfactants I. Selection of surfactant, variation of pH and addition of organic modifiers or inorganic metal salts

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Abstract

Although cationic surfactants offer complementary selectivity to anionic surfactants, most of the work in micellar electrokinetic chromatography (MEKC) has been done with anionic surfactants, due to the fact that the migration window is unfavorably much narrower with cationic surfactants than with anionic surfactants. In order to overcome the inherent disadvantages of cationic surfactants, methods are discussed and investigated that make it possible to widen the migration window. These methods include variation of the length of the alkyl chain of the surfactant employed, variation of the pH, addition of organic solvents and other organic modifiers, increase of the ionic strength of the separation electrolyte, and addition of inorganic salts with divalent metal ions ($CaCl_2$, $BaCl_2$). It will be shown that addition of inorganic salts with divalent metal ions can be successfully used in order to improve the migration window in MEKC with cationic surfactant Impact of the modifiers employed on retention of neutral and acidic solutes and on the efficiency of the separation system is investigated.

Keywords: Buffer composition; Elution window; Micellar electrokinetic chromatography; Benzaldehydes; Benzoic acids; Benzyl alcohols; Coumarin; Nitrotoluenes; Piperonal; Vanillic acid; Vanillin

1. Introduction

Micellar electrokinetic chromatography (MEKC), first introduced by Terabe and co-workers [1,2] renders possible the separation of neutral and charged solutes by distributing them between an aqueous mobile phase and a retarded micellar phase (pseudostationary phase) or by combining this separation mechanism with separation due to differences in the effective mobility of the solutes. The versatility and high efficiency of this method and its potential importance in routine analysis make it desirable that rapid and effective means of the optimization of the separation buffer are available. The optimization of separations performed with MEKC is complex and difficult due to the high number of parameters affecting the separation [3–5]. Further complications can arise from the mutual interaction of the parameters to be optimized.

One of the decisive parameters in MEKC is the selectivity of the separation system. In case of

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neutral solutes the selectivity is mainly dependent on the surfactant employed. Recently, characterization of surfactant selectivity in MEKC based on an understanding of the intramolecular interactions between the solutes and the micelles has been under intensive investigation [6–13]. These investigations include the application of linear solvation energy relationships and of the retention index concept.

It is desirable to have a set of surfactants with complementary selectivity. Poole and Poole [10] have highlighted that hexadecyltrimethylammonium bromide (CTAB) has a complementary selectivity to the other anionic surfactants employed in their study but provides only a small migration window (the ratio migration time of the micelles/hold-up time). Khaledi [4] reports that cationic micelles of CTAB have a very different interactive behavior than have micelles of sodium dodecyl sulfate. The CTAB micelles are stronger hydrogen bond acceptors. The main disadvantage of cationic surfactants, the small retention window, has already been reported by Otsuka et al. [14], who were the first, who employed a cationic surfactant as micelle forming agent in MEKC. They used dodecyltrimethylammonium bromide (DTAB).

In case of neutral solutes the migration window is directly related to the peak capacity [2]:

$$\mathrm{PK} = 1 + \frac{\sqrt{N}}{4} \cdot \ln \frac{t_{\mathrm{MC}}}{t_0} \tag{1}$$

where: PK = peak capacity, N = plate number, t_{MC} = elution time of the micelles, t_0 = hold-up time and to the resolution of closely eluting solutes [2]:

$$R = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k_{\mathrm{m}}}{1 + k_{\mathrm{m}}} \cdot \frac{1 - \frac{t_0}{t_{\mathrm{MC}}}}{1 + \frac{t_0}{t_{\mathrm{MC}}} k_{\mathrm{m}}} \tag{2}$$

where: R = resolution, α = selectivity and k_{m} = mean retention factor.

Currently, anionic surfactants are mostly preferred to cationic surfactants in MEKC [4,5]. In order to widen the range of suitable surfactant systems in MEKC, it would be highly desirable to improve the migration window when employing cationic surfactants, while the selectivity of the separation system is maintained.

For sodium dodecyl sulfate (SDS) as micelle forming agent it is known that the migration window can be greatly improved by reducing the pH of the separation electrolyte [15,16] or by adding appropriate modifiers [16–19]. In our study, methods are investigated that make it possible to widen the migration window when cationic surfactants are employed in order to overcome the disadvantages associated with this type of surfactants. These methods include variation of the length of the alkyl chain of the surfactant employed, variation of the pH, addition of organic solvents and other organic modifiers, increase of the ionic strength of the separation electrolyte and addition of inorganic salts to the separation electrolyte.

We also determined the critical micellar concentration (CMC) of alkyltrimethylammonium bromides with different chain length under conditions usually employed in MEKC in order to evaluate which surfactant meets best the requirements for MEKC concerning its physicochemical properties and the obtainable migration window.

2. Experimental

2.1. Standards and electrolyte components

Vanillic acid (4-hydroxy-3-methoxybenzoic acid), ethylvanillin (3-ethoxy-4-hydroxybenzaldehyde), 4-hydroxybenzyl alcohol, 4-methoxybenzoic acid and 2,4-dinitrotoluene were from Merck (Darmstadt, Germany), vanillin from Janssen (Brüggen, Germany), 4-hydroxy-3-methoxybenzyl alcohol, 3,4-dihydroxybenzoic acid, 2,3-dinitrotoluene, 2,5-dinitrotoluene, 2,6-dinitrotoluene and 3,4-dinitrotoluene from Aldrich (Steinheim, Germany), 4-hydroxybenzoic acid from Fluka (Neu-Ulm, Germany), trinitrotoluene from Promochem (Wesel, Germany). Thiourea, piperonal, coumarin, 4-hydroxybenzaldehyde and 4-methoxybenzaldehyde were available at the Department of Chemistry, University of Marburg. Sudan III was from Fluka (Buchs, Switzerland).

Sodium tetraborate, boric acid, CTAB, urea, dimethyl formamide, dimethyl sulfoxide, ethanol, calcium chloride dihydrate and tris(hydroxymethyl)aminomethane (Tris) were from Merck, tetradecyltrimethylammonium bromide (TTAB) from

Acros (NJ, USA), dodecyltrimethylammonium bromide (DTAB) from Fluka, acetic acid, barium chloride dihydrate, caesium chloride and potassium chloride from Riedel-de Haën (Seelze, Germany). Hydrochloric acid and sodium acetate trihydrate used for the preparation of the separation electrolytes were of analytical grade available at the Department of Chemistry, University of Marburg. Acetonitrile was distilled. Water was doubly distilled.

2.2. Preparation of buffers and standard solutions

Several buffers with equimolar content of buffer constituents have been employed to adjust the pH of the separation electrolyte. The pH values of 9.0, 7.0 and 4.6 correspond to the maxima of buffer capacity of the relevant buffer system. The pH of the buffer stock solutions was monitored by a Model 605 pH meter and an EA121 glass electrode (Metrohm, Herisau, Switzerland).

In case of constituents of the vanilla bean, sample solutions were prepared from stock solutions of the pure compound in water by diluting the appropriate volume of stock solution with distilled water (final concentration: 50–250 mg l^{-1}). In case of nitrotoluenes, the sample solutions are prepared in methanol–water (1:10, v/v).

2.3. Determination of the CMC

The CMC at 22.5°C of CTAB, TTAB and DTAB in buffers that have also been employed for MEKC separations were determined conductimetrically. Buffer solutions with varying concentrations of surfactant were prepared by the dilution of appropriate quantities of a surfactant stock solution in buffer with pure buffer.

The conductivity of the buffer in dependence on the surfactant concentration was determined with a Model E 365 B conductoscope (Metrohm) and two platinum electrodes. The temperature of the titration vessel was controlled before and after each titration. The CMC was determined from the inflection point of the resulting titration curve.

2.4. Chromatographic measurements

All chromatographic measurements were carried out with a Beckman (Fullerton, CA, USA) Model P/ACE capillary electrophoresis (CE) system equipped with a UV absorbance detector. The temperature of the capillary was controlled by liquid cooling and was maintained at 25°C. Samples were injected by application of pressure for 2 s. Detection was performed at 254 nm. All separations were carried out at a voltage of 15 or 25 kV. Data were recorded with the Beckman System Gold software.

Fused-silica capillaries (75 μm I.D.×375 μm O.D.) were obtained from Polymicro Technologies, Phoenix, AZ, USA. The total length of the capillary was 56.5 cm and the length to the detector was 50 cm. The elution time of the mobile phase, t_0, and the elution time of the micellar phase, t_{MC} were determined using thiourea and Sudan III, respectively, as markers. The repeatability of reported data has been confirmed by repeated injections. Presented data are the mean of at least two repeated measurements (relative standard deviation of elution time 0.1–0.2%). The significance of the impact of $CaCl_2$ as a modifier on the elution window has been confirmed by a second experiment employing a second fused-silica capillary, confirming the results of the first experiment (see Table 4).

3. Results and discussion

3.1. Selection of cationic surfactant and buffer system

In MEKC mostly alkyltrimethylammonium halides have been used as cationic surfactants. While CTAB as been the most popular surfactant used in CE, mainly to modify the capillary surface and to reverse the electroosmotic flow (EOF), also TTAB and DTAB have been used as micelle forming agents. These surfactants only differ in the length of the alkyl chain (see Fig. 1).

Surfactants employed in MEKC have to meet following requirements: low CMC, high solubility in aqueous solutions and low Krafft point (the temperature, under which the solubility is lower than the CMC). Crosby and El Rassi [20] evaluated a series of alkyltrimethylammonium chloride and bromide surfactants for MEKC. They report that the selectivity of the separation system for different homologous series was largely unaffected by the length of the alkyl group. The width of the migration window

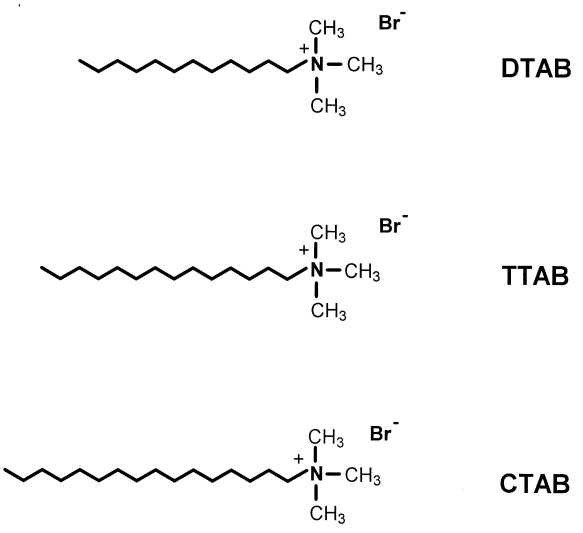

Fig. 1. Cationic surfactants employed.

increased with decreasing length of the alkyl chain of the surfactant.

In order to provide CMC data under conditions usually employed in MEKC, the CMC of DTAB, TTAB and CTAB was determined in various buffers applied in MEKC. It is well known that the CMC of ionic surfactants in buffers differs largely from the CMC determined in pure water [21,22]. It is known that the counter ion of the surfactant can affect the selectivity [23]. However, in case of cationic surfactants, it is impossible to use a buffer with an anion corresponding to the counter ion of the surfactant (as recommended with anionic surfactants). The following buffer systems have been selected for our studies: disodium tetraborate–boric acid (pH 9.0), Tris–hydrochloric acid (pH 7.0), sodium acetate–acetic acid (pH 4.6). The pH values of 9.0, 7.0 and 4.6 correspond to the maxima of buffer capacity of the relevant buffer system.

The CMC data obtained for these buffers by conductimetric titration (see Experimental) are listed in Table 1 and compared to values obtained in pure water. The comparison shows that the CMC of the cationic surfactants investigated is not in all cases strongly reduced compared to data reported for pure water in the literature. The CMC of TTAB in the buffers employed is in the same magnitude as the CMC of SDS in buffer solutions. The CMC of CTAB in buffer is one order of magnitude lower than the CMC of TTAB, while the CMC of DTAB exceeds 12 mmol l^{-1} in all cases. The CMC of the most popular surfactant used in MEKC, SDS at 20°C in a phosphate–borate buffer [$c(Na_2HPO_4)=10$ mmol l^{-1}, $c(Na_2B_4O_7)=10$ mmol l^{-1}] is 3.0 mmol l^{-1} [22], in the same magnitude as the values determined for TTAB in aqueous buffer solutions.

Concerning the CMC, DTAB is less suited for MEKC than TTAB, because separation electrolytes containing DTAB cause higher electric currents (resulting in Joule heating problems) than those containing TTAB at identical molar concentration due to the higher concentration of surfactant monomer in DTAB containing separation electrolytes. CTAB, however, has a very low solubility in aqueous solutions. Its solubility in water at 20°C is 9 mmol l^{-1}. Its use in MEKC ($c=10$–100 mmol l^{-1}) is therefore restricted to elevated temperatures ($T>30$°C). From the viewpoint of its physicochemical

Table 1
Critical micellar concentration (CMC) of alkyltrimethylammonium bromides at a temperature of 22.5°C in buffers used in MEKC at different pH (mean of two measurements)

Surfactant	CMC (mmol l^{-1})			
	pH 9.0[a]	pH 7.0[b]	pH 4.6[c]	(in water, 25°C)[d]
DTAB	14	13	16	16
TTAB	3.8	3.1	3.5	3.6
CTAB	0.75	0.40	0.37	0.92

[a] 10 mmol l^{-1} H_3BO_3, 10 mmol l^{-1} $Na_2B_4O_7$.
[b] 16 mmol l^{-1} HCl, 16 mmol l^{-1} Tris.
[c] 10 mmol l^{-1} CH_3COOH, 10 mmol l^{-1} $NaCH_3COO$.
[d] Data generated from Ref. [33].

properties, TTAB should be favored over its homologues.

3.2. Variation of pH

Cationic surfactants interact with the negatively charged silica capillary wall, form hemimicelles and reverse the direction of the EOF. Consequently, the polarity of the electrodes has to be reversed with respect to the polarity with anionic surfactants. The sample is injected at the cationic end and detection is performed at the anionic end. Due to the reversal of the EOF, the mobility of the micelles is directed opposite to the velocity of the aqueous phase as in the normal elution mode with anionic surfactant.

For a given surfactant the mobility of the micelles can be expected to be independent of the pH [24]. Hence, a decrease of the velocity of the aqueous phase would result directly in an improvement of the migration window. For MEKC with anionic surfactant in fused-silica capillaries it is known that the velocity of the EOF can be strongly reduced by reduction of the pH of the separation electrolyte [16].

In Table 2 the electroosmotic mobility, μ_{eo}, the electrophoretic mobility of the micelles μ_{MC} and the ratio t_{MC}/t_0 are given for the cationic surfactants employed at different surfactant concentrations and varied pH of the separation electrolyte. It can be seen that also at low pH the normal elution mode is maintained. The absolute mobility of the micelles $|\mu_{MC}|$ remains lower than the absolute electroosmotic mobility $|\mu_{eo}|$. These results corroborate the findings

Table 2
Electroosmotic mobility, μ_{eo}, electrophoretic mobility of the micelles, μ_{MC}, and the ratio t_{MC}/t_0 with the cationic surfactants employed under different surfactant concentrations c and varied pH (other conditions as in Table 1 and Fig. 2)

pH	Surfactant	c (mmol l^{-1})	μ_{eo} (10^{-3} cm^2 V^{-1} s^{-1})	μ_{MC} (10^{-3} cm^2 V^{-1} s^{-1})	t_{MC}/t_0
9.0	DTAB	40	−0.62	0.36	2.3
9.0	DTAB	70	−0.58	0.34	2.4
9.0	DTAB	100	−0.56	0.34	2.5
9.0	TTAB	40	−0.64	0.37	2.3
9.0	TTAB	70	−0.61	0.35	2.3
9.0	TTAB	100	−0.56	0.34	2.5
9.0	CTAB	40	−0.70	0.37	2.1
9.0	CTAB	70	−0.64	0.35	2.2
9.0	CTAB	100	−0.61	0.34	2.2
7.0	DTAB	40	−0.59	0.34	2.3
7.0	DTAB	70	−0.55	0.33	2.4
7.0	DTAB	100	−0.52	0.32	2.5
7.0	TTAB	40	−0.65	0.36	2.2
7.0	TTAB	70	−0.60	0.33	2.3
7.0	TTAB	100	−0.57	0.32	2.3
7.0	CTAB	40	−0.66	0.34	2.1
7.0	CTAB	70	−0.62	0.32	2.1
7.0	CTAB	100	−0.59	0.31	2.1
4.6	TTAB	40	−0.54	0.36	3.0
4.6	TTAB	70	−0.51	0.34	3.1
4.6	TTAB	100	−0.49	0.33	3.0
4.6	CTAB	40	−0.64	0.37	2.3
4.6	CTAB	70	−0.60	0.34	2.3
4.6	CTAB	100	−0.57	0.33	2.4

of Crosby and El Rassi [20] who state that with CTAB as surfactant the migration window stays practically unchanged as the pH of the separation electrolyte is varied between 4.5 and 9.0.

According to our results, there is, however, a decrease in the electroosmotic velocity with decreasing pH and with increasing surfactant concentration, resulting in a slight improvement of the migration window. At c(TTAB)=100 mmol l^{-1}, t_{MC}/t_0 is increased from 2.5 (pH=9.0) to 3.0 (pH=4.6). However, over the whole pH range investigated t_{MC}/t_0 is much lower than obtainable with an SDS-containing electrolyte under standard conditions {i.e., c(SDS)=100 mmol l^{-1}, $c(Na_2B_4O_7)$=10 mmol l^{-1}, $c(Na_2HPO_4)$=10 mmol l^{-1}, t_{MC}/t_0=4.3 [25]}. Consequently, with neutral solutes reduction of the pH

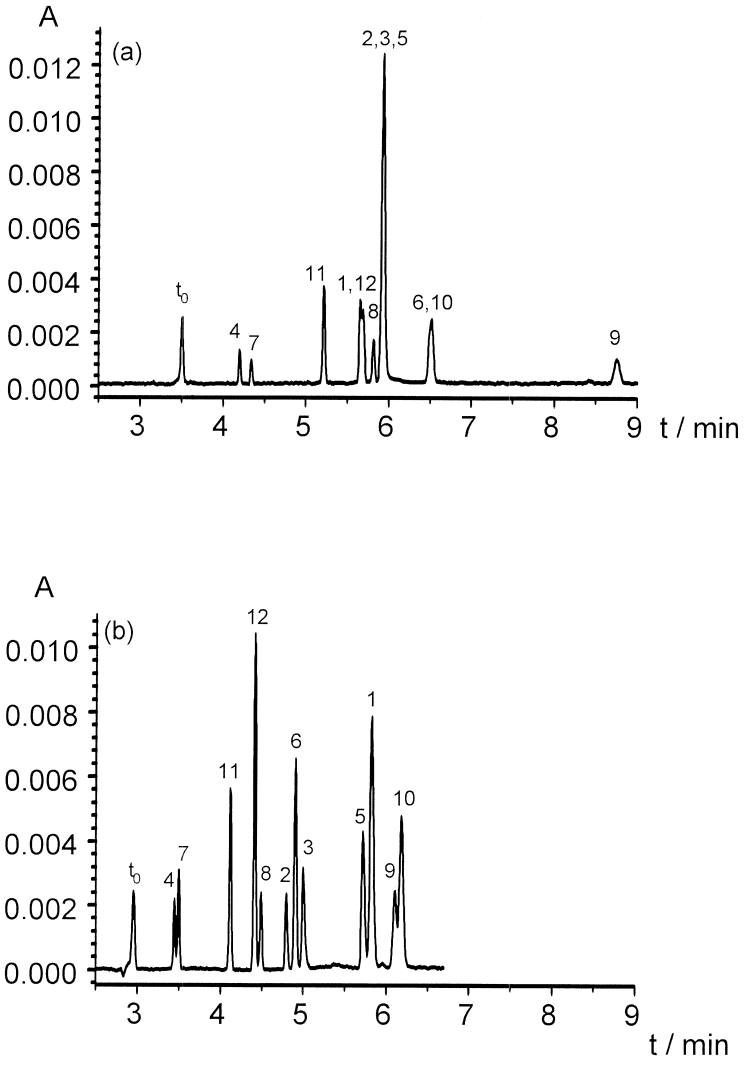

Fig. 2. Separation of neutral and acidic solutes at different pH [(a) 4.6, (b) 9.0] of the separation electrolyte. Electrolyte, TTAB=40 mmol l^{-1}, (a) $NaCH_3COO$=10 mmol l^{-1}, CH_3COOH=10 mmol l^{-1}, (b) H_3BO_3 10 mmol l^{-1}, $Na_2B_4O_7$ 10 mmol l^{-1}; capillary, 565 (500) mm×75 μm I.D.; voltage, 25 kV; temperature, 25°C; injection, pressure for 2 s; detection, UV at 254 nm. Peak identification: 1=vanillin; 2=vanillic acid; 3=3,4-dihydroxybenzoic acid; 4=4-hydroxy-3-methoxybenzyl alcohol; 5=4-hydroxybenzaldehyde; 6=4-hydroxybenzoic acid; 7=4-hydroxybenzyl alcohol; 8=4-methoxybenzaldehyde; 9=3-ethoxy-4-hydroxybenzaldehyde; 10=4-methoxybenzoic acid; 11=piperonal; 12=coumarin.

can be used for an improvement of the migration window, although even at a pH of 4.6 the migration window is much smaller than that obtainable with anionic surfactants under standard conditions.

With acidic or basic solutes change of the pH can completely alter the selectivity of the separation system due to secondary equilibria in aqueous solution and electrophoresis of the charged moieties. An example is given in Fig. 2 separating neutral and acidic solutes at different pH at constant surfactant concentration.

3.3. Alkyl chain length of the surfactant

The structure of the surfactant determines the aggregation number and the charge density of the micelles. It can be assumed therefore that the electrophoretic mobility of the micelles will depend strongly on the chain length of the surfactant. Increasing electrophoretic mobility of the micelles at constant velocity of the EOF will improve the migration window.

In Table 2 the electroosmotic mobility μ_{eo}, the electrophoretic mobility of the micelles μ_{MC} and the ratio t_{MC}/t_0 determining the migration window are given for alkyltrimethylammonium bromides of different alkyl chain length in dependence on the surfactant concentration and in dependence on the pH. There is an improvement in the migration window with decreasing length of the surfactant. This improvement is obviously due to an alteration of the hemimicelle structure in dependence on the surfactant employed. In contrast to our assumptions, the electrophoretic mobility of the micelles μ_{MC} is not affected significantly by the length of the alkyl chain of the surfactant. There is, however, a marked decrease in μ_{eo} with decreasing length of the alkyl chain (at constant pH and constant surfactant concentration). Therefore, from the aspect of improving the migration window a surfactant with short alkyl chain should be used in MEKC with cationic surfactant. The use of short chain surfactants, however, has the disadvantage of a high CMC (see Table 1) limiting the useful range of surfactants in MEKC. Considering the migration window TTAB should be preferred to CTAB.

Our results are in accordance with the findings of Crosby and El Rassi [20] who reported for alkyltrimethylammonium halides of different alkyl chain length employing a phosphate buffer (pH 7.0) that the width of the migration window increased with decreasing length of the alkyl chain of the surfactant. They attributed this improvement to an increase of the electrophoretic mobility of the micelles.

Table 3
Electroosmotic mobility, μ_{eo}, electrophoretic mobility of the micelles, μ_{MC}, the ratio t_{MC}/t_0, and the retention factors k of two selected neutral analytes in dependence on the content of an organic modifier (c_{mod} = concentration of modifier, φ_{mod} = volume fraction of modifier) in the separation electrolyte (40 mmol l^{-1} TTAB, 10 mmol l^{-1} H_3BO_3, 10 mmol l^{-1} $Na_2B_4O_7$, for other conditions see Fig. 2)

Modifier	c_{mod} (mol l^{-1})	φ_{mod} (%)	μ_{eo} (10^{-3} cm^2 V^{-1} s^{-1})	μ_{mc} (10^{-3} cm^2 V^{-1} s^{-1})	t_{MC}/t_0	k (Piperonal)	k (Coumarin)
Urea	0		−0.66	0.36	2.2	1.00	1.40
Urea	1		−0.65	0.36	2.2	0.76	1.03
Urea	2		−0.63	0.35	2.3	0.61	0.81
Urea	3		−0.60	0.34	2.3	0.50	0.66
Acetonitrile		0	−0.66	0.37	2.3	0.96	1.35
Acetonitrile		5	−0.64	0.36	2.3	0.77	1.02
Acetonitrile		10	−0.61	0.36	2.4	0.65	0.81
Acetonitrile		15	−0.60	0.36	2.5	0.51	0.61
Ethanol		0	−0.66	0.37	2.3	0.96	1.35
Ethanol		5	−0.55	0.31	2.3	0.79	1.05
Ethanol		10	−0.48	0.27	2.3	0.63	0.81
Ethanol		15	−0.40	0.24	2.4	0.51	0.62

3.4. Organic modifiers

Organic modifiers of the separation electrolyte have been used successfully in MEKC with anionic surfactants to reduce the distribution constant of neutral solutes between the micellar phase and the aqueous phase [16–19]. They can also have a dramatic effect on the migration window, i.e., urea has been employed successfully for an increase of t_{MC}/t_0 while the impact on the distribution coefficient for neutral solutes is relatively low [25,26]. At a concentration of SDS of 100 mmol l^{-1} t_{MC}/t_0 is improved from 4.3 [c(urea)=0 mol l^{-1}] to 7.7 [c(urea)=4 mol l^{-1}] employing a standard buffer [25], while the hold-up time t_o is not greatly affected due to anomalously low viscosities of aqueous urea solutions [27,28].

We have investigated whether those organic modifiers that have been used in MEKC with anionic surfactant can also be used in MEKC with cationic surfactant to adjust retention factors and to widen the retention window. Urea, acetonitrile, ethanol, di-

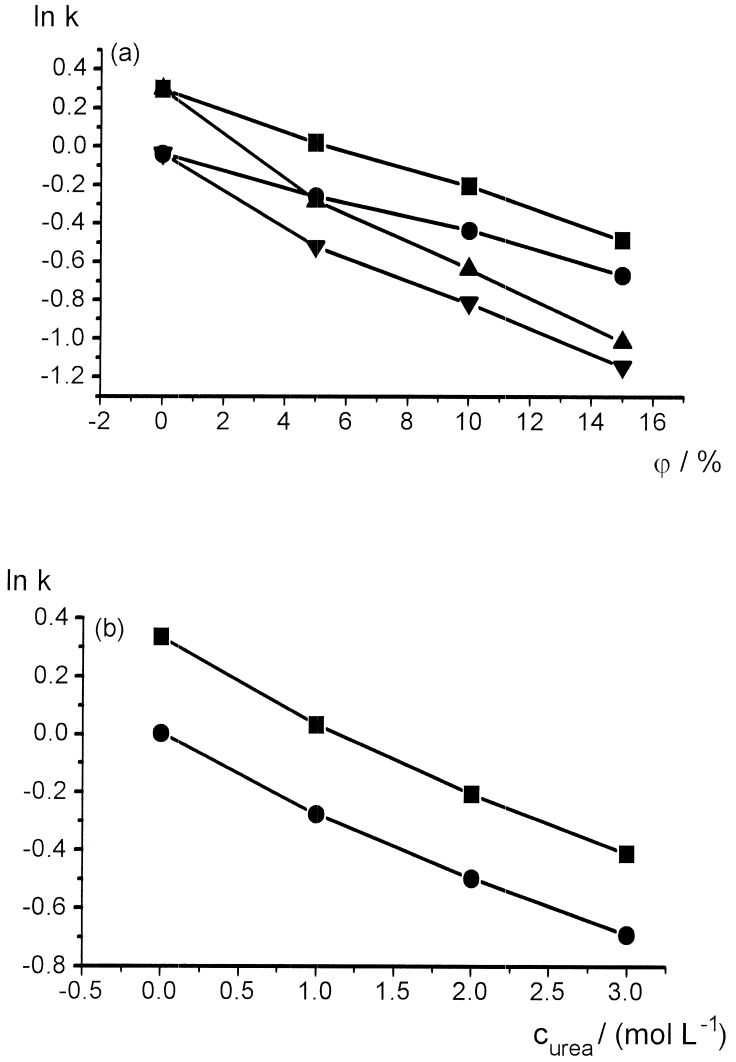

Fig. 3. Logarithm of retention factor for neutral solutes plotted against (a) volume fraction of acetonitrile (●=piperonal; ■=coumarin) or dimethylformamide (▼=piperonal; ▲=coumarin); (b) molar concentration of urea in the separation electrolyte (●=piperonal; ■=coumarin); for other conditions see Table 3.

Table 4
Electroosmotic mobility, μ_{eo}, electrophoretic mobility of the micelles μ_{McC}, the ratio t_{MC}/t_0, and the retention factors k of two selected neutral analytes in dependence on the concentration c_{mod} of an inorganic salt in the separation electrolyte, data of second experimental series for comparison in parentheses (40 mmol l^{-1} TTAB, buffers see Table 1, separation voltage = 15 kV, for other conditions see Fig. 2)

Modifier	pH	c_{mod} (mmol l^{-1})	μ_{eo} (10^{-3} cm^2 V^{-1} s^{-1})	μ_{MCl} (10^{-3} cm^2 V^{-1} s^{-1})	t_{MC}/t_0	k (Piperonal)	k (Coumarin)
KCl	4.6	0	−0.58	0.35	2.6	0.99	1.38
KCl	4.6	10	−0.54	0.34	2.7	0.98	1.37
KCl	4.6	30	−0.49	0.33	3.1	0.96	1.34
KCl	4.6	50	−0.46	0.33	3.4	0.94	1.31
KCl	9.0	0	−0.64	0.35	2.2	0.98	1.37
KCl	9.0	10	−0.59	0.35	2.4	0.98	1.36
KCl	9.0	30	−0.54	0.34	2.7	0.97	1.36
KCl	9.0	50	−0.52	0.34	2.9	0.95	1.33
$CaCl_2$	4.6	0	−0.58 (−0.57)	0.35 (0.35)	2.6 (2.6)	0.99 (0.98)	1.38 (1.37)
$CaCl_2$	4.6	10	−0.49 (−0.50)	0.34 (0.34)	3.2 (3.1)	0.97 (0.97)	1.35 (1.35)
$CaCl_2$	4.6	20	−0.45 (−0.46)	0.34 (0.33)	3.8 (3.6)	0.96 (0.96)	1.34 (1.34)
$CaCl_2$	4.6	30	−0.43 (−0.43)	0.33 (0.33)	4.5 (4.3)	0.95 (0.94)	1.32 (1.30)
$CaCl_2$	9.0	0	−0.61	0.35	2.4	0.98	1.37
$CaCl_2$	9.0	10	−0.54	0.33	2.6	–[a]	–[a]
$CaCl_2$	9.0	20	−0.51	0.34	2.9	0.98	1.36
$CaCl_2$	9.0	30	−0.48	0.33	3.2	0.99	1.38

[a] Not determined.

methyl formamide and dimethyl sulfoxide have been selected as suitable candidates. In Table 3 the electroosmotic mobility μ_{eo}, the electrophoretic mobility of the micelles μ_{MC}, the ratio t_{MC}/t_0, and the retention factors k of two selected neutral analytes are given for separation electrolytes containing TTAB (c = 40 mmol l^{-1}, pH 9.0) at different concentrations of the modifier (urea, acetonitrile and ethanol).

While the modifiers reduce the retention factors as described for anionic surfactants, the influence on the migration window is rather low. The same holds true for the modifiers dimethyl formamide and dimethyl sulfoxide (volume fraction of modifier in separation electrolyte = 0–15%) under the conditions listed in Table 3. In no case there is an increase in μ_{MC} affected by addition of a modifier. Improvement of t_{MC}/t_0 is only achieved by reduction of μ_{eo}. Possibly, there is an alteration of the hemimicelle structure in presence of organic modifiers. Increase in viscosity of the electrolyte (i.e., for addition of ethanol to the separation electrolyte) is reflected in a decrease in μ_{MC} and μ_{eo}.

In Fig. 3 the logarithm of the retention factors for two selected neutral analytes are plotted against the volume fraction φ of the modifier acetonitrile or dimethyl formamide or against the concentration of modifier urea in the separation electrolyte. For acetonitrile and ethanol the dependence can be described by the function:

$$\ln k = a + b\varphi \quad (3)$$

where: k = retention factor, a, b = constants, φ = volume fraction of modifier.

This relationship is well-known for reversed-phase high-performance liquid chromatography [29] and for MEKC [16,25,26]. For the modifiers dimethyl formamide, dimethyl sulfoxide and urea, however, there is a deviation from the linearity for the dependence of the logarithm of the retention factor for neutral solutes from the volume fraction (or molar concentration) of the modifier.

3.5. Metal cations

There are CE methods that use zinc or barium salts to improve the resolution [30]. In these cases the selectivity was not affected but the electroosmotic velocity was decreased. Also Pietrzyk et al. [31]

report that inorganic cations can be used as additives to separation electrolytes to control the EOF. They employed Mg^{2+}, Zn^{2+} and Cd^{2+} salts. Brechtel et al. [30] state that Ba^{2+} salts are effective additives to suppress the EOF even at high pH. Measurements of the streaming potential of quartz have shown that inorganic counter ions change the electrokinetic potential ζ [32]. Multivalent inorganic counter ions are even able to change the sign of ζ. These measurements have also shown that surface inactive cations compete with cationic surfactant monomers for positions in the electric double layer.

We studied whether the addition of inorganic salts with monovalent or divalent cations can be used to reduce the electroosmotic velocity in MEKC with cationic surfactants, assuming that the cations compete with the surfactant monomers for ion-exchange positions on the fused-silica surface, hence reducing the charge density of the formed hemimicelles. CsCl, KCl, $CaCl_2$ and $BaCl_2$ have been selected as suitable

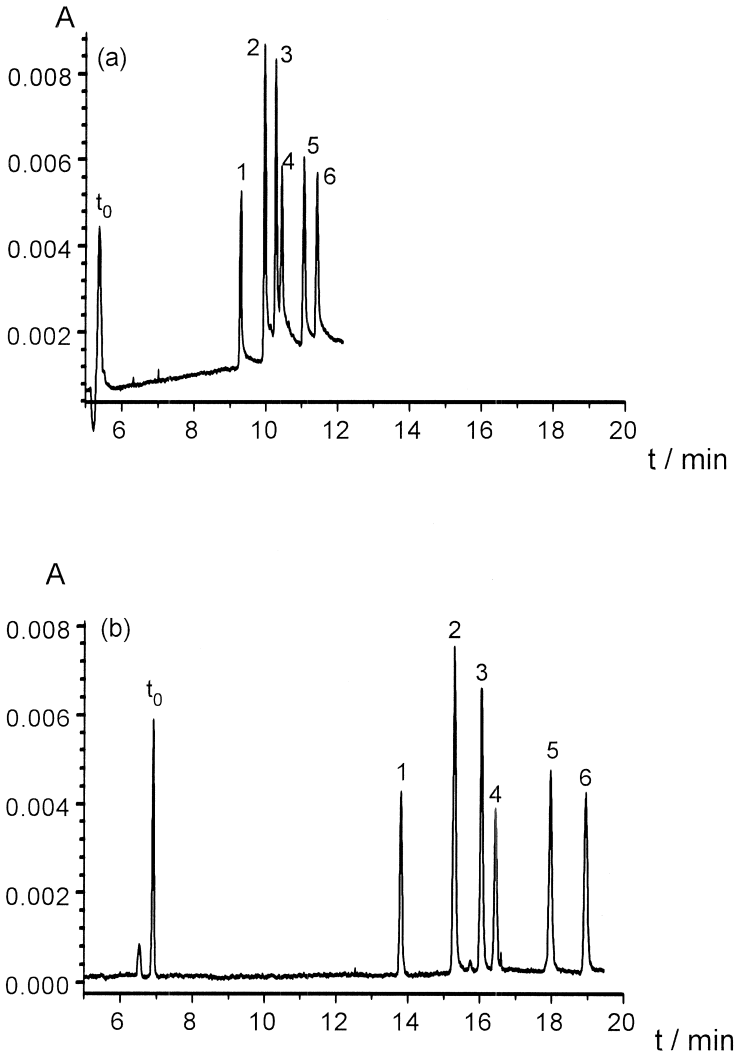

Fig. 4. Separation of nitrotoluenes with a separation electrolyte containing (a) 0 mmol l^{-1}, (b) 30 mmol l^{-1} $CaCl_2$ (peak identification: 1=2,4,6-trinitrotoluene, 2=2,5-dinitrotoluene, 3=2,4-dinitrotoluene, 4=2,6-dinitrotoluene, 5=3,4-dinitrotoluene, 6=2,3-dinitrotoluene; for other conditions see Table 4).

additives. If the electroosmotic velocity is reduced while the electrophoretic mobility of the micelles is maintained, the migration window can be expanded.

In Table 4 the electroosmotic mobility μ_{eo}, the electrophoretic mobility of the micelles μ_{MC}, the ratio t_{MC}/t_0, and the retention factors k of two selected neutral analytes are given for separation electrolytes (pH 9.0 or 4.6) containing TTAB ($c=40$ mmol l^{-1}) at different concentrations of the additives KCl and $CaCl_2$. A disadvantage of inorganic salts as additives is an increase in the electric current during a chromatographic run due to a strong increase in the specific conductivity of the separation electrolyte. I.e., by addition of 30 mmol l^{-1} $CaCl_2$ to an electrolyte containing 40 mmol l^{-1} TTAB, 10 mmol l^{-1} $Na_2B_4O_7$, and 10 mmol l^{-1} H_3BO_3 with the capillary dimensions given in Experimental, at a separation voltage of 25 kV the electric current strength recorded during separation was increased from 59 μA to 200 μA. In order to avoid overheating, the separation voltage had to be reduced form 25 kV to 15 kV.

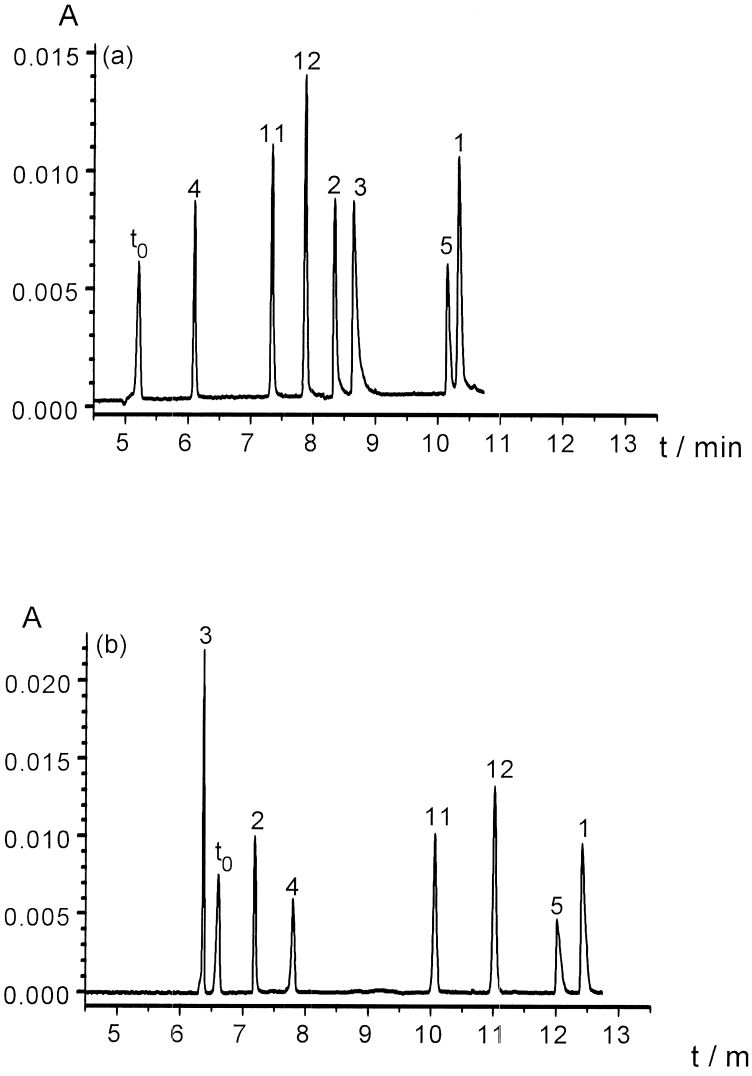

Fig. 5. Separation of neutral and acidic solutes with a separation electrolyte (pH=9.0) containing (a) 0 mmol l^{-1}, (b) 30 mmol l^{-1} $CaCl_2$ (for peak identification see Fig. 2, for other conditions see Table 4).

According to the theory, that metal cations are incorporated into the hemimicelles, thus decreasing the electrokinetic potential at the inner capillary wall, there is a decrease in the electroosmotic mobility μ_{eo} with increasing content of metal cation in the separation electrolyte. The decrease in μ_{eo} is more pronounced with divalent cations than with monovalent cations. Ba^{2+} and Ca^{2+} do not differ in their ability to reduce μ_{eo}. The same holds true for the cations Cs^{+} and K^{+} (data not shown). Obviously, at first order the radius of the solvated cation does not influence the impact of the modifier on μ_{eo}.

At pH 9.0 by addition of 30 mmol l^{-1} $CaCl_2$ or $BaCl_2$, respectively, the ratio t_{MC}/t_0 is improved from 2.4 to 3.2; at pH 4.6 by addition of 30 mmol l^{-1} $CaCl_2$ or $BaCl_2$, respectively, the ratio t_{MC}/t_0 is improved from 2.6 to 4.5. The decrease in μ_{eo} is not compensated by a decrease in the mobility of the micelles. The electrophoretic mobility of the micelles is at first order independent of the pH and of the concentration of the modifier. The retention factors for neutral solutes are slightly decreased by the addition of inorganic salts. This decrease is probably due to the increase in the specific conductivity of the separation electrolyte resulting in an increase in temperature during the separation process.

We also examined whether this improvement in t_{MC}/t_0 can be obtained alternatively by an increase in the ionic strength of the separation electrolyte. Borate–boric acid buffers (pH 9.0) with a molar concentration of each component of 5 to 25 mmol l^{-1} have been employed with constant surfactant (TTAB) concentration ($c=40$ mmol l^{-1}). The improvement in t_{MC}/t_0 is in this case much lower. The ratio t_{MC}/t_0 is only increased from 2.1 to 2.3.

In Fig. 4 the MEKC separation of nitrotoluenes with a separation electrolyte (pH=4.6) containing 40 mmol l^{-1} TTAB is shown and compared to a separation with the same electrolyte containing additionally 30 mmol l^{-1} Ca^{2+}. The decrease in μ_{eo} caused by addition of $CaCl_2$ is reflected by the increase in t_0 resulting in a longer run time (12 min→20 min). With help of the modifier the resolution of closely eluting peaks is greatly improved. The efficiency of the separation system is maintained. The plate number calculated for the peaks shown in Fig. 4b is 210 000–240 000. The elution order is not affected by the modifier.

The selectivity for neutral solutes is not altered by the addition of inorganic salts. However, there is a strong impact on the retention of acidic solutes. An example is given in Fig. 5 separating neutral and acidic solutes at constant surfactant concentration at pH=9.0 with and without addition of $CaCl_2$ at a concentration of 30 mmol l^{-1}. The retention of negatively charged solutes is strongly decreased. 3,4-

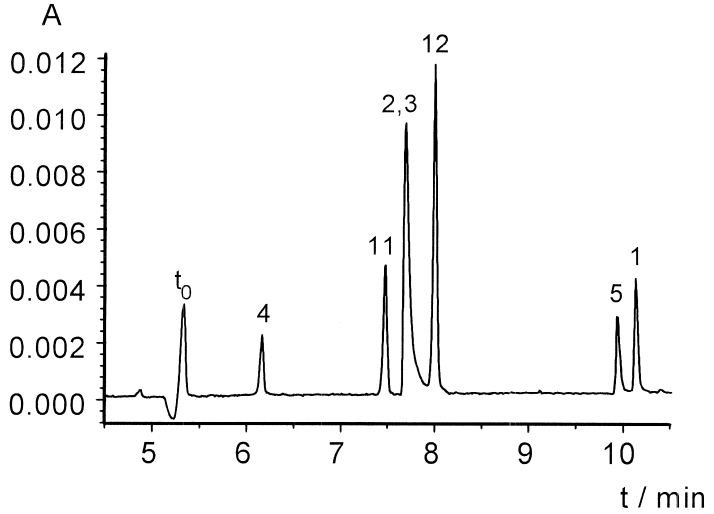

Fig. 6. Separation of neutral and acidic solutes with a separation electrolyte (pH=9.0) containing 25 mmol l^{-1} H_3BO_3, 25 mmol l^{-1} $Na_2B_4O_7$ (for peak identification see Fig. 2, for other conditions see Table 4).

Dihydroxybenzoic acid is even eluted in front of thiourea serving as an EOF marker. Obviously, the increase in the concentration of metal cations in the separation electrolyte results in a decrease in the distribution coefficients between the micellar and the aqueous phase for negatively charged solutes. Reduction of the retention for negatively charged solutes is also observed when the ionic strength of the separation electrolyte is increased (Fig. 6, to be compared to Fig. 5a).

If a separation electrolyte buffered at lower pH (4.6) is employed, the dissociation of weak organic acids is suppressed. Consequently, there should be no alteration of the elution order for solutes with $pK_S >> 4.6$ by addition of an inorganic salt to the separation electrolyte. This prediction could be experimentally verified (Fig. 7). At the lower pH of the separation electrolyte only the retention for the benzoic acids (vanillic acid, 3,4-dihydroxybenzoic acid) is strongly decreased by addition of an inorganic salt. Obviously, the increase in the ionic strength affects only the interaction of negatively charged species with the positively charged micelles, which are mainly electrostatic in nature. In case of

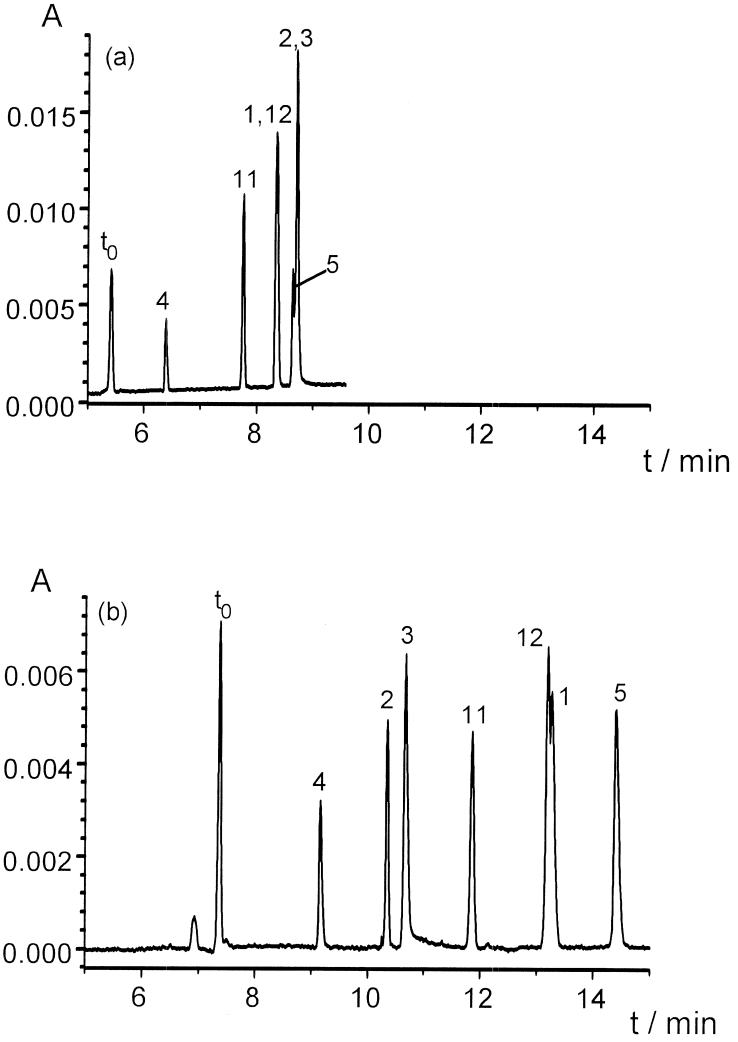

Fig. 7. Separation of neutral and acidic solutes with a separation electrolyte (pH = 4.6) containing (a) 0 mmol l^{-1}, (b) 30 mmol l^{-1} $CaCl_2$ (for peak identification see Fig. 2, for other conditions see Table 4).

neutral solutes, small changes in the retention factor can be attributed to temperature effects.

4. Conclusions

Concerning physicochemical properties and the migration window obtained TTAB should be preferred to its homologues DTAB and CTAB.

Not all of the tested measures result in a sufficient widening of the migration window in MEKC with cationic surfactant. A reduction of the pH of the separation electrolyte has only a small influence on the electroosmotic mobility μ_{eo}, hence does not improve the ratio t_{MC}/t_0 in a sufficient manner. Also the organic modifiers employed do not improve the migration window substantially, although they can be employed for adjusting of retention factors.

By addition of inorganic salts to the separation electrolyte a strong decrease in the electroosmotic mobility μ_{eo} and a substantial improvement of the migration window was obtained, especially at low pH of the separation electrolyte. The decrease in μ_{eo} is more pronounced with divalent cations than with monovalent cations.

Addition of inorganic salts to the separation electrolyte does not alter the chromatographic selectivity for non-charged solutes but reduces strongly the retention of acidic solutes (in dependence on the pH of the separation electrolyte). Hence, inorganic salts can be employed as modifiers to improve the resolution in MEKC with cationic surfactant. In the case of negatively charged solutes, the impact of the modifier on the separation selectivity has to be taken into consideration.

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References

[1] S. Terabe, K. Otsuka, K. Ichkaea, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111.
[2] S. Terabe, K. Otsuka, T. Ando, Anal. Chem. 57 (1985) 834.
[3] H. Corstjens, H.A.H. Billiet, J. Frank, K.C.A.M. Luyben, J. Chromatogr. A 715 (1995) 1.
[4] M.G. Khaledi, J. Chromatogr. A 780 (1997) 3.
[5] P.G. Muijselaar, K. Otsuka, S. Terabe, J. Chromatogr. A 780 (1997) 41.
[6] S. Yang, M.G. Khaledi, J. Chromatogr. A 692 (1995) 301.
[7] P.G. Muijselaar, H.A. Claessens, C.A. Cramers, Anal. Chem. 66 (1994) 635.
[8] S. Yang, M.G. Khaledi, Anal. Chem. 67 (1995) 499.
[9] S. Yang, J.G. Baumgarner, M.G. Khaledi, J. Chromatogr. A 738 (1996) 265.
[10] S.K. Poole, C.F. Poole, Analyst 122 (1997) 267.
[11] P.G. Muijselaar, H.A. Claessens, C.A. Cramers, Anal. Chem. 69 (1997) 1184.
[12] P.G. Muijselaar, H.A. Claessens, C.A. Cramers, Chromatographia 45 (1997) 433.
[13] C.F. Poole, S.K. Poole, M.H. Abraham, J. Chromatogr. A 798 (1998) 207.
[14] K. Otsuka, S. Terabe, T. Ando, J. Chromatogr. 332 (1985) 219.
[15] H.T. Rasmussen, H.M. McNair, J. High Resolut. Chromatogr. 12 (1989) 635.
[16] P.G.H.M. Muijselaar, H.A. Claessens, C.A. Cramers, J. Chromatogr. A 696 (1995) 273.
[17] J. Gorse, A.T. Balchunas, D.F. Swaile, M.J. Sepaniak, J. High Resolut. Chromatogr. Chromatogr. Commun. 1 (1988) 554.
[18] U. Pyell, U. Bütehorn, J. Chromatogr. A 716 (1995) 81.
[19] U. Bütehorn, U. Pyell, J. Chromatogr. A 772 (1997) 27.
[20] D. Crosby, Z. El Rassi, J. Liq. Chromatogr. 16 (1993) 2161.
[21] D.A. Armstrong, Sep. Purif. Methods 14 (1985) 213.
[22] U. Pyell, U. Bütehorn, Chromatographia 40 (1995) 69.
[23] K.R. Nielsen, J.P. Foley, J. Microcol. Sep. 5 (1993) 347.
[24] K. Otsuka, S. Terabe, J. Microcol. Sep. 1 (1989) 347.
[25] U. Pyell, U. Bütehorn, Chromatographia 40 (1995) 175.
[26] S. Terabe, Y. Ishihama, H. Nishi, T. Fukuyama, K. Otsuka, J. Chromatogr. 545 (1991) 359.
[27] R. Muckerjee, A. Ray, J. Phys. Chem. 67 (1963) 190.
[28] M.J. Schick, J. Phys. Chem. 68 (1964) 3585.
[29] L.R. Snyder, J. Chromatogr. B 689 (1997) 105.
[30] R. Brechtel, W. Hohmann, H. Rüdiger, H. Wätzig, J. Chromatogr. A 716 (1995) 97.
[31] D.J. Pietrzyk, S. Chen, B. Chanthawat, J. Chromatogr. A 755 (1997) 327.
[32] D.W. Fuerstenau, J. Phys. Chem. 60 (1956) 981.
[33] M.J. Rosen, in: Surfactants and Interfacial Phenomena, 2nd ed, Wiley, New York, 1989, pp. 108–142.