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Widening of the elution window in micellar electrokinetic chromatography with cationic surfactants II. Cationic additives and modifiers of the electroosmotic flow

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Abstract

In micellar electrokinetic chromatography (MEKC) with cationic surfactants the migration window is significantly narrower than with anionic surfactants. In order to overcome this disadvantage of cationic surfactants, it is investigated whether it is possible to widen the migration window by reducing the velocity of the aqueous phase while the electrophoretic mobility of the micelles is maintained. Short chain alkylammonium compounds, hexamethonium bromide and hydroxypropylmethylcellulose are tested as additives to the separation electrolyte with the potential to improve the migration window via reducing the velocity of the electroosmotic flow. It will be shown that these modifiers can be successfully used in order to widen the migration window in MEKC with cationic surfactant employing an alkyltrimethylammonium bromide as micelle forming agent. Influence of the modifiers selected on retention of neutral and acidic solutes and on efficiency of the separation system is investigated.

Keywords: Electroosmotic flow; Micellar electrokinetic chromatography; Buffer composition; Surfactants; Nitrotoluenes; Piperonal; Coumarin; Vanillin

1. Introduction

Optimization of separations performed with micellar electrokinetic chromatography (MEKC) is complex and difficult due to the high number of parameters affecting the separation [1–3] and their mutual interaction. In the case of neutral solutes the selectivity of the separation system is mainly dependent on the surfactant employed. 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 [4–11]. In the case of ionizable solutes also other factors have to be taken into account due to a separation process combining partition between phases of different velocity and electrophoretic migration.

In order to provide tools for a rapid optimization of the composition of the separation electrolyte, it is desirable to have a set of surfactants with complementary selectivity. Several authors have highlighted that cationic surfactants provide a complementary selectivity to anionic surfactants but chromatographic runs show only a small migration

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window (the ratio migration time of the micelles/hold-up-time) [2,8,12].

In the case of neutral solutes the migration window is directly related to the peak capacity [13]:

$$PK = 1 + \frac{\sqrt{N}}{4} \cdot \ln \frac{t_{MC}}{t_0} \quad (1)$$

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 [13]:

$$R = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k_m}{1 + k_m} \cdot \frac{1 - \frac{t_0}{t_{MC}}}{1 + \frac{t_0}{t_{MC}} k_m} \quad (2)$$

where: R = resolution, α = selectivity, k_m = mean retention factor.

Disadvantages associated with the use of cationic surfactants in MEKC concerning peak capacity and resolution of closely eluting solutes make these type of surfactants unfavourable compared to anionic surfactants. In the present literature, in MEKC anionic surfactants are mostly preferred to cationic surfactants [2,3]. The objective of this work was to investigate how the migration window can be improved in MEKC with cationic surfactant, without altering the selectivity of the separation system.

In our previous article [14] we have already given a brief overview of how the migration window can be improved when employing an anionic surfactant in MEKC. We have also studied whether variation of the pH, addition of organic solvents and other modifiers, increase of the ionic strength or the addition of inorganic salts with divalent cations can be successfully employed in MEKC with cationic surfactants in order to increase the ratio migration time of the micelles/hold-up-time. An increase of this ratio can be attained by either (1) increasing the electrophoretic mobility of the micelles while keeping the electroosmotic mobility constant or (2) reducing the electroosmotic mobility while keeping the electrophoretic mobility of the micelles constant. In our previous study [14] we have shown that the addition of inorganic salts with divalent cations to the separation electrolyte reduces significantly the electroosmotic mobility, thus rendering it possible to widen the migration window. There is, however, a strong impact on the separation selectivity with acidic solutes at high pH (9.0) of the separation electrolyte.

In this paper, we have studied whether other additives competing with the surfactant monomers for positions in the hemimicelles formed at the surface of the inner capillary wall can be used to reduce the velocity of the aqueous phase while the electrophoretic mobility of the micelles is maintained. Hexamethonium bromide (HMB), tetrabutylammonium bromide (TBAB) and octyltrimethylammonium bromide (OTAB) are investigated as potential candidates. Another possibility to reduce the electroosmotic mobility is dynamic coating of the capillary wall with a suitable polymer. In this study, hydroxypropylmethyl cellulose has been used for a dynamic coating of the inner capillary surface.

We studied the impact of the additives selected on the migration window, the retention of neutral and acidic solutes, the selectivity and the efficiency of the separation system.

2. Experimental

2.1. Standards and electrolyte components

Vanillic acid (4-hydroxy-3-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), 2,4,6-trinitrotoluene from Promochem (Wesel, Germany). Thiourea, piperonal, coumarin, and 4-hydroxybenzaldehyde were available at the Department of Chemistry, University of Marburg. Sudan III was from Fluka (Buchs, Switzerland).

Sodium tetraborate, boric acid, and TBAB were from Merck (Darmstadt, Germany), tetradecyltrimethylammonium bromide (TTAB) from Acros (NJ, USA), OTAB from Fluka (Buchs, Switzerland), acetic acid from Riedel-de Haën (Seelze, Germany), hexamethonium bromide (hexane-1,6-bis-trimethylammonium bromide) from Aldrich, hydroxypropylmethylcellulose (2% sol., 4000 CP) from Sigma (St.

Louis, MO, USA). Sodium acetate trihydrate used for the preparation of the separation electrolytes was of analytical grade available at the Department of Chemistry, University of Marburg. Water was doubly distilled.

2.2. Preparation of buffers and standard solutions

Several buffers with equimolar content of buffer constituents (maximum of buffer capacity) have been employed to adjust the pH of the separation electrolyte. The pH of the buffer stock solutions was controlled by a Model 605 pH meter and an EA121 glass electrode (Metrohm, Herisau, Switzerland).

In the 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 the case of nitrotoluenes, the sample solutions are prepared in methanol–water (1:10, v/v).

2.3. Chromatographic measurements

All chromatographic measurements were carried out with a Beckman (Fullerton, CA, USA) model P/ACE capillary electrophoresis 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. Peak identities were confirmed by spiking. 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%).

3. Results and discussion

3.1. Selection of cationic surfactant, buffer system and test solutes

In our previous article [14] we have shown that from the viewpoint of its physicochemical properties, TTAB should be favoured to its homologues in MEKC with cationic surfactant. Therefore, all measurements have been performed exclusively with TTAB.

The pH of the separation electrolyte has been adjusted with a borate–boric acid buffer (pH 9.0) or with an acetate–acetic acid buffer (pH 4.6). It is inevitable in MEKC with cationic surfactant that the counter ions of the formed micelles (here bromide ions) are in part replaced by the anions of the buffer system selected.

In order to study the influence of the modifiers on the selectivity of the separation system, constituents of extracts of the vanilla bean (neutral compounds and weak organic acids) and several nitrotoluenes (neutral compounds) have been selected.

3.2. Short chain alkyl ammonium compounds

Nishi et al. [15] employed tetraalkylammonium salts as selectivity modifying additives in MEKC with the anionic surfactant sodium dodecylsulfate (SDS). According to Nishi et al. the positively charged tetraalkylammonium ions combine with the negatively charged organic sulfate ions and alter the character of the micelles.

In the case of MEKC with cationic surfactant no ion pair interaction of the tetraalkylammonium ions with the positively charged micelles can be expected. Long-chain tetraalkylammonium ions might be incorporated in the micelles forming mixed micelles with the cationic surfactant. Strong electrostatic interaction, however, is only possible with the negatively charged dissociated silanol groups of the capillary wall. These are shielded by a double layer of cationic surfactant (the hemimicelle). If the tetraalkylammonium ions are incorporated into the double layer of cationic surfactant the resulting charge density on the capillary wall will be reduced resulting in a decrease of the electroosmotic velocity. Due to differences in the chemical structure, the

space demand of an adsorbed tetraalkylammonium (i.e. tetrabutylammonium) ion will be higher than that of a rod-like alkyltrimethylammonium ion. If the electrophoretic mobility of the micelles is not influenced by the addition of tetraalkylammonium salts to the separation electrolyte, the addition of tetraalkylammonium salts should result in an improvement of the migration window in MEKC with cationic surfactant.

Crosby and El Rassi [16] recommended to use a short alkyl chain surfactant OTAB as additive to separation electrolytes containing a cationic surfactant for enlarging the migration window. According to their theory OTAB is incorporated into the micelles, forming mixed micelles with surfactant molecules of a larger alkyl chain. The critical micelle concentration (CMC) of this short chain surfactant measured in pure water at 25°C is 140 mmol l^{-1} [16].

In order to investigate whether tetraalkylammonium salts or short chain surfactants are suitable modifiers in MEKC with cationic surfactant, the following additives have been selected: TBAB and OTAB. The concentration of TBAB or OTAB is varied from 0–100 mmol l^{-1}. Because of the high content of electrolyte in these solutions the separation voltage had to be restricted to 15 kV in order to avoid overheating due to Joule heat formation.

In Table 1 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 (piperonal and coumarin) are given for separation electrolytes (pH = 9.0 or 4.6) containing TTAB ($c = 40$ mmol l^{-1}) at different

Table 1
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 pH and concentration c of modifier OTAB or TBAB in the separation electrolyte (40 mmol l^{-1} TTAB; pH 9.0: 10 mmol l^{-1} H_3BO_3, 10 mmol l^{-1} $Na_2B_4O_7$; pH 4.6: 10 mmol l^{-1} CH_3COOH, 10 mmol l^{-1} $NaCH_3COO$; other conditions see Fig. 1)

Modifier	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	k (piperonal)	k (coumarin)
OTAB, pH 9.0	0	−0.64	0.35	2.2	0.97	1.36
	20	−0.52	0.31	2.4	0.93	1.29
	40	−0.45	0.29	2.7	0.94	1.29
	60	−0.40	0.28	3.3	0.92	1.26
	80	−0.37	0.26	3.5	0.92	1.25
	100	−0.34	0.25	3.7	0.95	1.29
OTAB, pH 4.6	0	−0.56	0.35	2.7	1.00	1.40
	20	−0.47	0.31	2.9	0.94	1.23
	40	−0.41	0.29	3.3	0.91	1.17
	60	−0.36	0.27	4.0	0.92	1.15
	80	−0.32	0.26	5.6	0.88	1.08
	100	−0.28	–[a]	–[a]	–[a]	–[a]
TBAB, pH 9.0	0	−0.64	0.35	2.2	1.00	1.41
	20	−0.52	0.31	2.4	0.92	1.27
	40	−0.46	0.28	2.5	0.89	1.21
	60	−0.42	0.26	2.6	0.84	1.15
	80	−0.39	0.25	2.8	0.82	1.10
	100	−0.36	0.24	2.9	0.78	1.04
TBAB, pH 4.6	0	−0.54	0.35	2.9	1.00	1.40
	20	−0.43	0.30	3.5	0.93	1.22
	40	−0.37	0.28	3.9	0.88	1.13
	60	−0.34	0.25	4.2	0.85	1.07
	80	−0.31	0.24	4.4	0.83	1.03
	100	−0.29	–[a]	–[a]	–[a]	–[a]

[a] Not measured.

concentrations of the additives. Independent of the pH, there is a strong decrease in the electroosmotic mobility with increasing content of TBAB or OTAB, respectively, in the separation electrolyte. There is also a decrease in the electrophoretic mobility of the micelles with increasing concentration of additive, possibly due to an increase in the viscosity of the separation electrolyte or due to the formation of mixed micelles. Although the decrease in μ_{MC} partly compensates the effect of the decrease in μ_{eo}, the alteration in the mobilities results in a strong improvement of the migration window. At pH 9.0 the ratio t_{MC}/t_0 is improved from 2.2 to 2.9 by addition of 100 mmol l^{-1} TBAB and to 3.7 by addition of 100 mmol l^{-1} OTAB. At pH 4.6 the ratio t_{MC}/t_0 is improved from 2.9 to 4.4 by addition of 80 mmol l^{-1} TBAB and to 5.6 by addition of 80 mmol l^{-1} OTAB. The improvement in t_{MC}/t_0 is more pronounced with OTAB than with TBAB. This improvement cannot be ascribed to an increase in μ_{MC}. It is presumably due to an alteration of the structure of the hemimicelles formed on the inner capillary wall/aqueous phase interphase.

The retention factors for the neutral solutes selected are reduced with increasing content of modifier in the separation electrolyte. This effect is observed to a much lower extent with addition of inorganic salts to the separation electrolyte [14]. In the case of inorganic salts as additives this effect might be due to an increase in the conductivity of the separation electrolyte resulting in an increase in the electric current during the separation, hence increasing the separation temperature. The more pronounced effect with OTAB or TBAB as additives suggests that these additives reduce also the distribution constant between the micellar phase and the aqueous phase, possibly via the formation of mixed micelles with the cationic surfactant.

In Fig. 1(a) and (b) the MEKC separation of nitrotoluenes with a separation electrolyte (pH = 4.6)

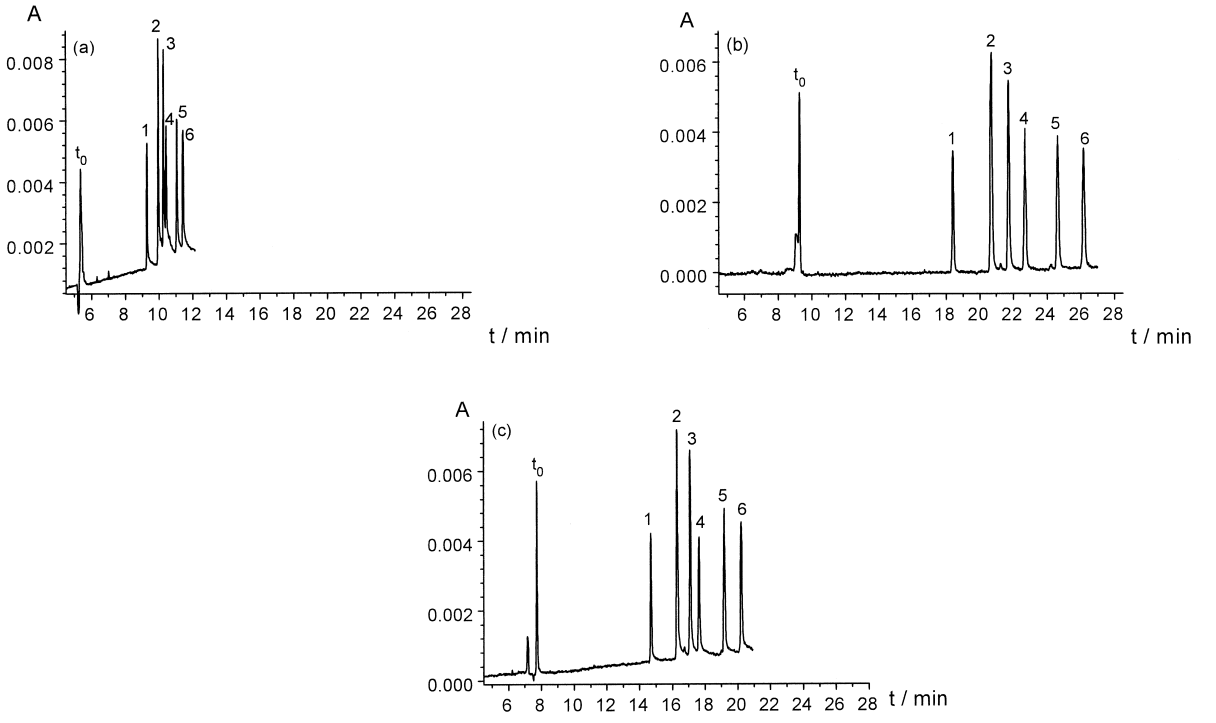

Fig. 1. Separation of nitrotoluenes with a separation electrolyte (TTAB = 40 mmol l^{-1}, $NaCH_3COO$ = 10 mmol l^{-1}, CH_3COOH = 10 mmol l^{-1}, pH 4.6) containing (a) no modifier (b) 100 mmol l^{-1} OTAB, (c) 40 mmol l^{-1} HMB. Capillary, 565 (500) mm × 75 μm I.D.; voltage, 15 kV; temperature, 25°C; injection, pressure for 2 s; detection, UV at 254 nm. 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.

containing 40 mmol l^{-1} TTAB is compared to a separation with the same electrolyte containing additionally 100 mmol l^{-1} OTAB. The decrease in μ_{eo} caused by addition of OTAB is reflected by the increase in t_0 (5→9 min) resulting in a longer run time. With the 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. 1(b) is 112 000–160 000. The elution order is not affected by the modifier.

The additives employed did not alter the selectivity for neutral solutes, but they have a strong impact on the selectivity for acidic solutes. An example is given in Fig. 2 separating neutral and acidic solutes at constant surfactant concentration. The separation of these solutes with a separation electrolyte (pH 9.0) containing 40 mmol l^{-1} TTAB is shown and compared to separations with electrolytes containing additionally 40 or 100 mmol l^{-1} OTAB. By addition of the modifier the retention of partially negatively charged solutes (vanillic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzaldehyde, vanillin) is strongly decreased depending on the concentration of modifier. At 100 mmol l^{-1} OTAB the solutes vanillic acid and 3,4-dihydroxybenzoic acid are even eluted in front of the EOF-marker. The same effect on the selectivity was observed for TBAB as modifier (results not shown).

At pH 4.6 the dissociation of the acidic solutes with $pK_a \gg 4.6$ is suppressed. Hence only the retention of solutes with lower pK_a values (vanillic acid, $pK_a = 4.52$ (25°C, in water) [17]; 3,4-dihydroxybenzoic acid, $pK_a = 4.48$ (25°C, in water) [18]) is strongly affected by the addition of TBAB or OTAB to the separation electrolyte (see Fig. 3(a) and (b)), while the elution order for the other less acidic compounds (phenolic compounds and neutral com-

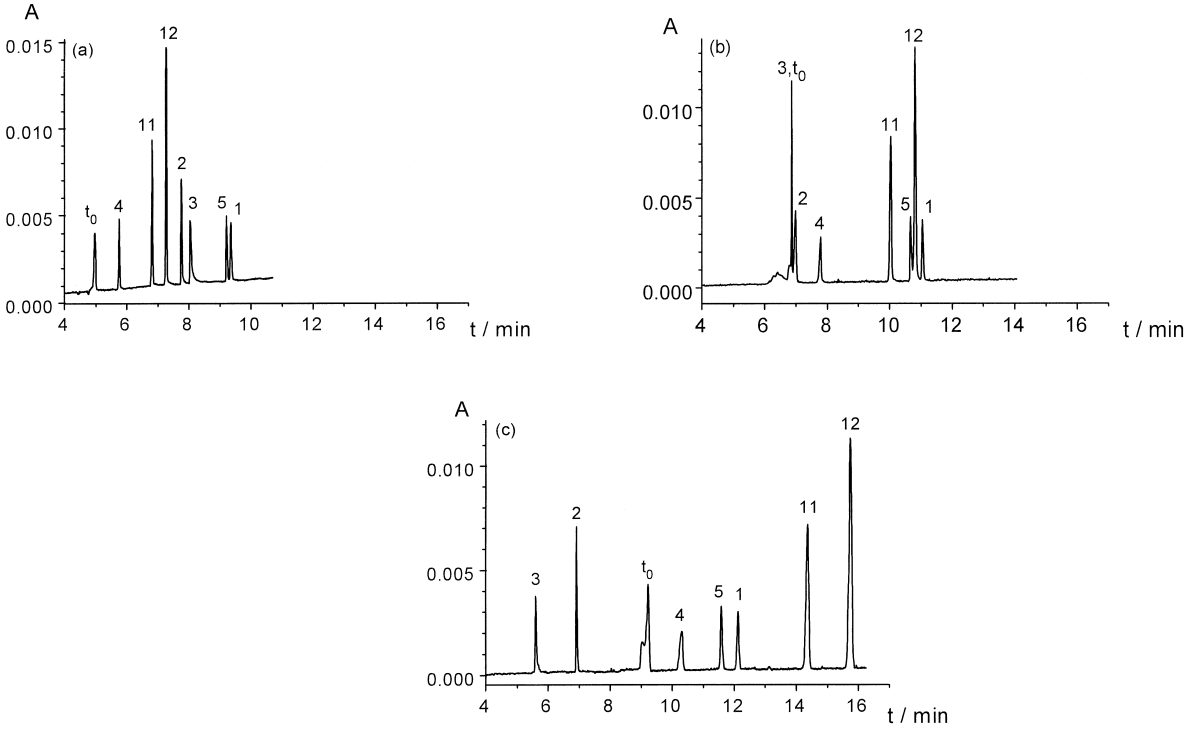

Fig. 2. Separation of neutral and acidic solutes with a separation electrolyte (TTAB = 40 mmol l^{-1}, $Na_2B_4O_7$ = 10 mmol l^{-1}, H_3BO_3 = 10 mmol l^{-1}, pH 9.0) containing (a) 0 mmol l^{-1}, (b) 40 mmol l^{-1}, (c) 100 mmol l^{-1} OTAB (other conditions see Fig. 1). Peak identification: 1 = vanillin; 2 = vanillic acid; 3 = 3,4-dihydroxybenzoic acid; 4 = 4-hydroxy-3-methoxybenzyl alcohol; 5 = 4-hydroxybenzaldehyde; 11 = piperonal; 12 = coumarin.

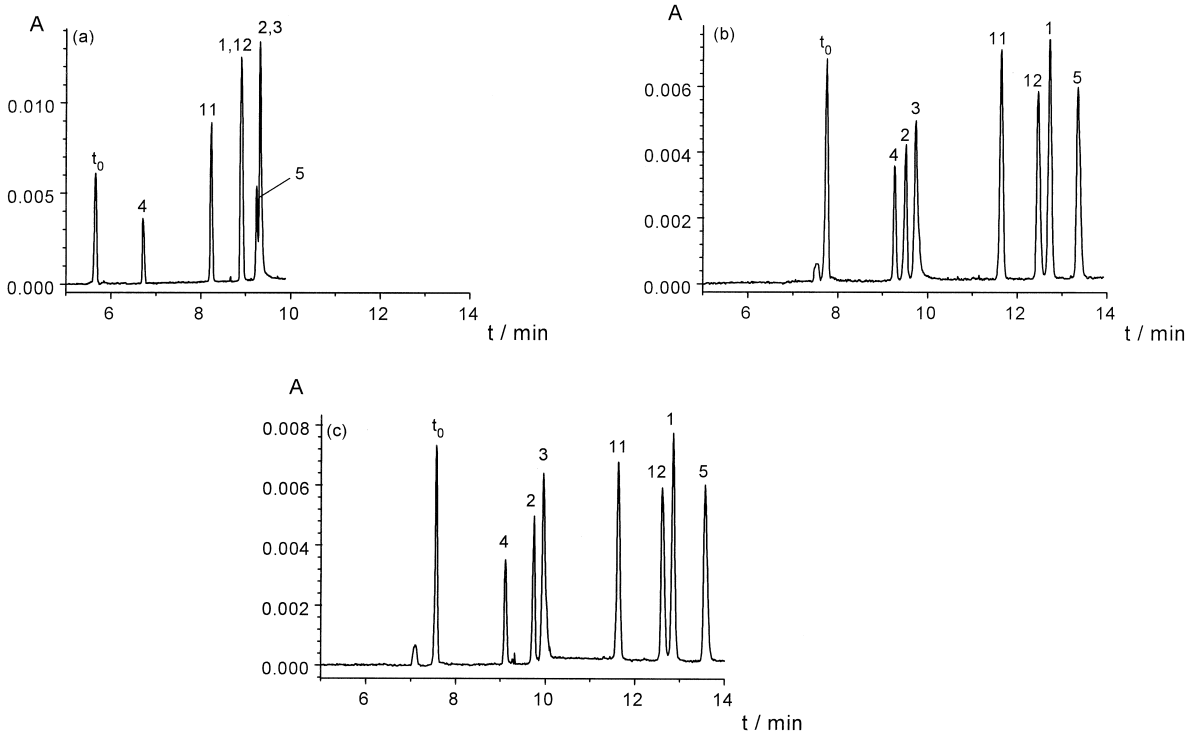

Fig. 3. Separation of neutral and acidic solutes with a separation electrolyte (TTAB = 40 mmol l^{-1}, $NaCH_3COO$ = 10 mmol l^{-1}, CH_3COOH = 10 mmol l^{-1}, pH 4.6) containing (a) no modifier, (b) 40 mmol l^{-1} OTAB, (c) 20 mmol l^{-1} HMB (other conditions see Fig. 1, peak identification see Fig. 2).

pounds, pK_a (phenol) = 9.89 (25°C, in water) [18]) is kept constant. At pH 9.0, however, also the retention of phenolic solutes (vanillin, 4-hydroxybenzaldehyde) was substantially reduced by the cationic additives tested, resulting in alterations of the elution order at higher modifier concentration (see Fig. 2).

In our previous paper [14] we observed a similar effect on the retention of neutral and acidic solutes caused by an increase of the ionic strength of the separation electrolyte. An increase in the ionic strength selectively reduces the retention of partly anionic solutes while the retention of neutral solutes is not affected. Obviously, the increase in the ionic strength affects only the interaction of negatively charged species with the positively charged micelles, which is mainly electrostatic in nature. In the case of neutral solutes, the small changes in the retention factor observed with increase in the ionic strength can be attributed to temperature effects.

3.3. Hexamethonium bromide

Hexane-1,6-bis-trimethylammonium bromide (hexamethonium bromide, HMB) has been used as an electroosmotic flow (EOF) modifier in the capillary electrophoretic separation of anions [19]. It strongly interacts with the negatively charged dissociated silanol groups of the capillary wall.

We studied whether the addition of this EOF modifier can be used to reduce the electroosmotic velocity in MEKC with cationic surfactants, assuming that HMB competes with the surfactant monomers for ion-exchange positions on the fused-silica surface, hence reducing the charge density of the formed hemimicelles. If it is possible to reduce the electroosmotic velocity while the electrophoretic mobility of the micelles is maintained, an improvement in the ratio t_{MC}/t_0 will result.

In Table 2 the electroosmotic mobility μ_{eo}, the

Table 2
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 pH and concentration c of modifier hexamethonium bromide in the separation electrolyte (40 mmol l^{-1} TTAB; pH 9.0: 10 mmol l^{-1} H_3BO_3, 10 mmol l^{-1} $Na_2B_4O_7$; pH 4.6: 10 mmol l^{-1} CH_3COOH, 10 mmol l^{-1} $NaCH_3COO$; other conditions see Fig. 1)

pH	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	k (piperonal)	k (coumarin)
9.0	0	−0.63	0.35	2.2	1.02	1.42
	10	−0.55	0.33	2.5	0.96	1.33
	20	−0.50	0.31	2.7	0.94	1.32
	30	−0.46	0.30	2.9	0.90	1.25
4.6	0	−0.54	0.35	2.9	1.00	1.39
	10	−0.46	0.33	3.5	0.93	1.29
	20	−0.42	0.31	3.9	0.89	1.24
	30	−0.39	0.30	4.6	0.86	1.20
	40	−0.36	0.29	5.3	0.84	1.16

electrophoretic mobility of the micelles μ_{MC}, the ratio t_{MC}/t_0, and the retention factors k of two selected neutral analytes (piperonal and coumarin) are given for separation electrolytes (pH = 9.0 or 4.6) containing TTAB (c = 40 mmol l^{-1}) and different concentrations of the EOF-modifier HMB. According to our theory, independent of the pH, there is a strong decrease in the electroosmotic mobility with increasing content of HMB. However, there is also a decrease in the electrophoretic mobility of the micelles with increasing concentration of additive. This decrease in μ_{MC} partly counteracts the effect of the decrease in μ_{eo}. In spite of these counteracting effects, the alteration in the mobilities results in an improvement of the migration window. At pH 9.0 the ratio t_{MC}/t_0 is improved from 2.2 to 3.1 by addition of 40 mmol l^{-1} HMB and at pH 4.6 the ratio t_{MC}/t_0 is improved from 2.9 to 5.3.

Comparing the potential of HMB for improving the migration window in MEKC with cationic surfactant to other additives tested in this work and in our previous paper [14]: inorganic salts with divalent metal cations, TBAB, and OTAB; HMB does not offer distinct advantages over the other additives tested.

The impact of HMB on the separation selectivity follows the tendencies already found for other cationic additives. The retention factors for neutral solutes are reduced with increasing content of modifier in the separation electrolyte (see Table 2). The selectivity for neutral solutes is not altered. In Fig. 1(a) and (c) the MEKC separation of nitrotoluenes with a separation electrolyte (pH = 4.6) containing 40 mmol l^{-1} TTAB is compared to a separation with the same electrolyte containing additionally 40 mmol l^{-1} HMB. With the help of the modifier HMB 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. 1(c) is 200 000–218 000. The elution order is not affected by the modifier.

The increase in the ionic strength associated with the addition of HMB to the separation electrolyte has a strong impact on the retention of acidic solutes. In Fig. 4 the impact on the separation of neutral and acetic solutes by addition of HMB to the separation electrolyte is shown. The separation of these solutes with a separation electrolyte (pH 9.0) containing 40 mmol l^{-1} TTAB is compared to separations with electrolytes containing additionally 20 or 40 mmol l^{-1} HMB. As already observed with OTAB and TBAB the retention of partially negatively charged solutes (vanillic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzaldehyde, vanillin) is strongly decreased depending on the concentration of modifier resulting in alterations of the elution order.

This decrease in retention can be suppressed for the phenolic compounds, if the separation electrolyte is buffered at lower pH (see Fig. 3(a) and (c)), supporting our theory that the alteration in selectivity

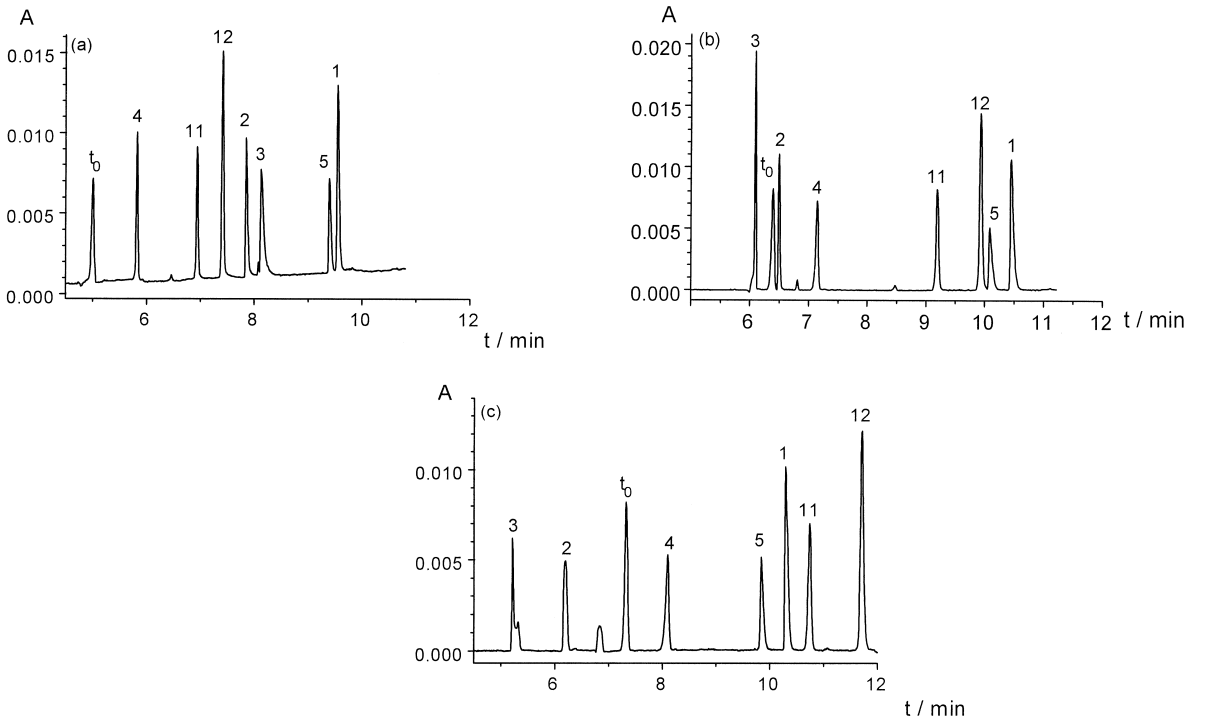

Fig. 4. Separation of neutral and acidic solutes with a separation electrolyte (TTAB = 40 mmol l^{-1}, $Na_2B_4O_7$ = 10 mmol l^{-1}, H_3BO_3 = 10 mmol l^{-1}, pH 9.0) containing (a) 0 mmol l^{-1}, (b) 20 mmol l^{-1}, (c) 40 mmol l^{-1} HMB (other conditions see Fig. 1, peak identification see Fig. 2).

is due to changes affecting the anionic species/cationic micelle interaction, that is mainly electrostatic in nature.

3.4. Hydroxypropylmethyl cellulose

Muijselaar et al. [20] discuss widening of the migration window in MEKC with anionic surfactant by modification of the capillary surface. Capillaries coated by polyethylene glycol showed a decrease in the electroosmotic mobility but also a decrease in the efficiency that was ascribed to solute–wall interactions. They conclude that the application of coated capillaries is of limited use in MEKC.

Hydroxypropylmethylcellulose (HPMC) is a modifier that is used to reduce or even suppress the EOF in fused-silica capillaries [21]. In our studies, HPMC was used to reduce the electroosmotic mobility in MEKC separations with cationic surfactant. Assuming that the electrophoretic mobility of the micelles will not be altered by a dynamic coating of the capillary walls, a widening of the migration window can be obtained.

Preliminary studies investigating the repeatability of separations have shown that drastic rinsing procedures have to be performed to avoid irreproducible results. Between runs the capillary was rinsed for 0.5 min with NaOH (c = 0.1 mol l^{-1}) and 1.5 min with a separation electrolyte containing HPMC. The separation was then performed with an electrolyte containing no HPMC, so that HPMC was only used to pre-condition the capillary. By additional measurements we were able to show that with rinsing the capillary for 4 min with HCl (c = 1 mol l^{-1}) after having finished the separation HPMC can be completely removed.

In Table 3 the impact of pre-conditioning the capillary with HPMC (0.0001%, m/v, dissolved in separation electrolyte) on the electroosmotic mobility μ_{eo}, the electrophoretic mobility of the micelles μ_{MC},

Table 3
Influence of the dynamic coating by HPMC on electroosmotic mobility μ_{eo}, electrophoretic mobility of the micelles μ_{MC}, and the ratio t_{MC}/t_0 (80 mmol l^{-1} TTAB; pH 9.0: 10 mmol l^{-1} H_3BO_3, 10 mmol l^{-1} $Na_2B_4O_7$; other conditions see Fig. 5)

Coating	μ_{eo} (10^{-3} cm^2 V^{-1} s^{-1})	μ_{MC} (10^{-3} cm^2 V^{-1} s^{-1})	t_{MC}/t_0
No pre-treatment	−0.63	0.35	2.2
Pre-treated with 0.0001% (m/v) HPMC	−0.49	0.35	3.5

and the ratio t_{MC}/t_0 is given for a separation electrolyte (pH 9.0) containing 80 mmol l^{-1} TTAB. The electroosmotic mobility can be strongly reduced by the dynamic coating procedure employed. As μ_{MC} is not effected, the migration window can be improved, reflected by an increase in t_{MC}/t_0 from 2.2 to 3.5.

In Fig. 5 the separation of neutral and acidic compounds [pH 9.0, c(TTAB)=80 mmol l^{-1}] with and without pre-conditioning of the capillary with HPMC are compared. While the selectivity of the separation system is maintained, the migration window is widened at the cost of longer analysis time. The retention factors for the neutral and acidic solutes are not affected by the conditioning procedure. The major disadvantage of the dynamic coating procedure employed is a strong decrease in efficiency. The plate numbers for the separation without dynamic coating are in the range 150 000–330 000. with the HPMC-coated capillary only plate numbers in the range 40 000–80 000 are obtained. The improvement in resolution by widening of the migration window is fully compensated by the reduced efficiency. There is no improvement in resolution for closely eluted solutes. The observed decrease in efficiency with dynamically coated capillaries is probably due to solute–wall interactions.

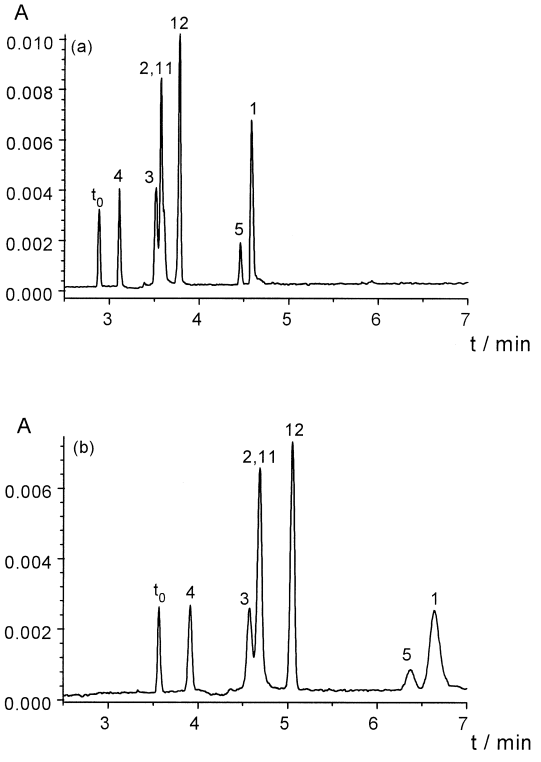

Fig. 5. Separation of neutral and acidic solutes (a) without (b) after pre-conditioning of the capillary with HPMC (0.0001%, m/v). Separation electrolyte: TTAB=80 mmol l^{-1}, $Na_2B_4O_7$=10 mmol l^{-1}, H_3BO_3=10 mmol l^{-1}, pH 9.0; voltage, 25 kV; other conditions see Fig. 1, peak identification see Fig. 2.

4. Conclusions

The migration window in MEKC with cationic surfactant can be improved by decreasing the electroosmotic velocity while the electrophoretic mobility of the micelles is maintained. HMB, TBAB and OTAB are suitable modifiers improving the migration window in MEKC with cationic surfactant (tetradecyltrimethylammonium bromide). Best results have been obtained with OTAB. The improvement in t_{MC}/t_0 obtained with OTAB as a modifier is even larger than with the addition of inorganic salts with divalent metal cations investigated in a previous paper [14]. The major disadvantage of ionic modifiers is an increase in the specific conductivity of the separation electrolyte limiting the maximum separation voltage. According to our theory, the modifiers employed alter the structure of the hemimicelles formed on the inner capillary surface by competing

with the surfactant monomers for ion-exchange positions on the fused-silica surface.

The modifiers employed reduce strongly the retention of negatively charged moieties interacting with the positively charged micelles. This effect on the retention of acidic solutes can be used for fine-tuning the selectivity of the separation system.

Dynamic coating of the capillaries by HPMC is of limited use in MEKC with cationic surfactants due to a strong decrease in efficiency of the separation system observed with dynamically coated capillaries.

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