Migration behaviour of monovalent weak acids in micellar electrokinetic chromatography
Mobility model versus retention model

Pim G. Muijselaar*, Henk A. Claessens, Carel A. Cramers

Laboratory of Instrumental Analysis, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, Netherlands

Abstract

Migration of charged compounds in micellar electrokinetic chromatography (MEKC) is based on micellar solubilization as well as electrophoretic migration. Consequently, the migration behaviour of these compounds can be described with a retention model or a mobility model. Both models are discussed and evaluated for the migration behaviour of monovalent weak acids in a sodium dodecyl sulphate micellar system. We have shown that the calculation of retention factors may be troublesome for hydrophobic compounds, due to interaction with surfactant molecules in the aqueous phase. Therefore, the mobility model is preferable. The simultaneous determination of dissociation constants by spectroscopic and electrophoretic methods is discussed and apparent dissociation constants in micellar media are determined, showing that pK_a shifts may occur in MEKC analyses, due to micellar solubilization. Furthermore, a sample stacking procedure is described, based on differences in overall effective mobilities between aqueous and micellar electrolyte systems.

Keywords: Retention model; Mobility model; Micellar electrokinetic chromatography; Acids; Weak acids, monovalent

1. Introduction

Micellar electrokinetic chromatography (MEKC) is a highly efficient analytical technique [1,2], not only suitable for the separation of neutral compounds, but also for mixtures of charged and uncharged compounds [3,4] and for charged compounds with almost identical electrophoretic mobilities [5,6]. The migration behaviour of charged compounds in MEKC is based on partitioning with the pseudo-stationary micellar phase as well as electrophoretic migration in the aqueous phase. A

good understanding of the influence of experimental conditions on these partitioning and migration processes would greatly facilitate the development of optimization strategies for complex sample mixtures. Recently, the migration behaviour has been studied extensively and several models have been reported that describe migration in terms of physico-chemical constants, such as micelle binding constants and apparent dissociation constants in micellar media [7–11].

In this work, the mobility and retention models for monovalent weak acids in an anionic micellar system are evaluated and compared. The determination of apparent dissociation constants in aqueous and micellar media by electrophoretic and spectroscopic

---

*Corresponding author. Present address: Faculty of Science, Himeji Institute of Technology, Kamigori, Hyogo 678-12, Japan.

methods is examined simultaneously. In addition, a sample stacking procedure, based on mobility differences due to micellar solubilization, is discussed.

2. Theory

2.1. Migration parameters

The separation mechanism of charged compounds in MEKC is based on both chromatographic and electrophoretic principles. Therefore, the migration of these compounds can be described in terms of the retention factor, i.e., a chromatographic parameter, or in terms of mobility, i.e., an electrophoretic parameter. Khaledi et al. [7] reported in detail a phenomenological approach to describe the migration behaviour of monovalent acidic compounds as a function of buffer pH and micelle concentration. In this approach, the net migration parameter of an acidic compound is assumed to be the weighted average of the migration parameter of the acid in the undissociated and the dissociated form, in the aqueous and the micellar phase, respectively. Thus, the overall effective mobility, m_{eff}^{ov}, in a micellar electrolyte system is given by:

$$m_{eff}^{ov} = \frac{1}{1 + k} m_{eff,AQ} + \frac{k}{1 + k} m_{MC}$$ (1)

where k is the retention factor, $m_{eff,AQ}$ is the effective mobility of the acid in the aqueous phase and m_{MC} is the effective mobility of the micellar phase. The overall effective mobility can be calculated directly from the migration times of the electrokinetic chromatogram according to:

$$m_{eff}^{ov} = \frac{l_c l_d}{t_S V} - \frac{l_c l_d}{t_{EOF} V}$$ (2)

where l_c is the length of the capillary, l_d is the length from injection to detection, t_S is the migration time of the solute, t_{EOF} is the migration time of the electroosmotic flow (EOF), and V is the applied voltage.

2.2. Retention factor

From Eq. (1), the following expression for the retention factor can be derived:

$$k = \frac{m_{eff}^{ov} - m_{eff,AQ}}{m_{MC} - m_{eff}^{ov}}$$ (3)

The effective mobility in the aqueous phase, $m_{eff,AQ}$, is a difficult parameter from MEKC experiments. Therefore, effective mobilities, obtained with capillary zone electrophoresis (CZE) experiments, are often used in this equation. However, it should be noted that several assumptions are made in this case, e.g., the influence of the micelles on ionic strength, dielectric constant and viscosity, and consequently on the effective mobility, are assumed to be negligible and interactions of sample compounds with surfactant monomers are assumed not to occur. The retention factor as a function of pH is expressed by [7]:

$$k = \frac{k_{HA} + k_{A^-} (K_a/[H^+])}{1 + K_a/[H^+]}$$ (4)

Eq. (4) predicts a sigmoidal behaviour of k versus pH, identical to that of liquid chromatographic techniques, with k_{HA} and k_{A^-} as the limiting retention factors of the acid in the undissociated and the dissociated form, respectively.

2.3. Overall effective mobility

The effective mobility of an acid in the aqueous phase, $m_{eff,AQ}$, is given by:

$$m_{eff,AQ} = \frac{K_a}{K_a + [H^+]} m_{A^-,AQ}$$ (5)

where $m_{A^-,AQ}$ is the effective mobility of the fully dissociated acid in the aqueous phase.

The apparent dissociation constant in micellar media, $K_{a,app}$, is defined by [7,12]:

$$K_{a,app} = K_a \frac{K_{A^-}^m [M] + 1}{K_{HA}^m [M] + 1}$$ (6)

where K_{HA}^m and $K_{A^-}^m$ are the binding constants to the micelles of the undissociated and the dissociated form of the acid, respectively, and [M] is the concentration of surfactant, present as micelles. Combination of Eqs. (1,4–6) leads to the following expression for the overall effective mobility as a function of pH [7]:

$$m_{\text{eff}}^{\text{ov}} = \frac{m_{\text{HA}} + m_{\text{A}^-} (K_{\text{a,app}}/[\text{H}^+])}{1 + K_{\text{a,app}}/[\text{H}^+]} \qquad (7)$$

with

$$m_{\text{HA}} = \frac{K_{\text{HA}}^m [\text{M}] m_{\text{MC}}}{1 + K_{\text{HA}}^m [\text{M}]} \qquad (8)$$

and

$$m_{\text{A}^-} = \frac{m_{\text{A}^-,\text{AQ}} + K_{\text{A}^-}^m [\text{M}] m_{\text{MC}}}{1 + K_{\text{A}^-}^m [\text{M}]} \qquad (9)$$

Eq. (7) predicts a sigmoidal behaviour of $m_{\text{eff}}^{\text{ov}}$ versus pH, with m_{HA} and m_{A^-} as the limiting mobilities of the acid in the undissociated and the dissociated form in micellar media, respectively.

2.4. Spectroscopic pK_a determination

According to Beer's law, the absorbance, A, for a UV detector equals:

$$A = \epsilon \, c \, l \qquad (10)$$

where ϵ is the molar absorptivity, c is the concentration of the UV absorbing species and l is the effective path length. For a monovalent acid at a concentration of c_{HA}, the absorbance at a specific wavelength will be:

$$A = [(1 - \alpha) \, \epsilon_{\text{HA}} + \alpha \, \epsilon_{\text{A}^-}] \, c_{\text{HA}} \, l \qquad (11)$$

where ϵ_{HA} and ϵ_{A^-} are the molar absorptivities of the acid in the undissociated and the dissociated form, respectively, and α is the degree of dissociation.

Generally, in capillary electrophoretic techniques, peak areas are expressed on a temporal basis [13]. On applying a non-UV-absorbing electrolyte system, the measured temporal peak area, A_{T}, will be proportional to:

$$A_{\text{T}} \propto Q_{\text{inj}} [(1 - \alpha) \, \epsilon_{\text{HA}} + \alpha \, \epsilon_{\text{A}^-}] \, t_{\text{S}} \qquad (12)$$

where Q_{inj} is the injected sample amount. From Eq. (12) it can be concluded that, for a constant sample amount, the spatial peak area A_{S}, i.e., the temporal peak area multiplied by the migration velocity, is proportional to:

$$A_{\text{S}} \propto \frac{\epsilon_{\text{HA}} + \epsilon_{\text{A}^-} (K_{\text{a}}/[\text{H}^+])}{1 + K_{\text{a}}/[\text{H}^+]} \qquad (13)$$

Eq. (13) predicts a sigmoidal behaviour of A_{S} versus pH. This expression can be applied for the spectroscopic determination of dissociation constants in aqueous media, pK_a, by CZE experiments [14] or apparent dissociation constants in micellar media, $pK_{\text{a,app}}$, by MEKC experiments, provided that the molar absorptivities ϵ_{HA} and ϵ_{A^-} differ sufficiently at the applied wavelength. Notice that for micellar media K_a should be replaced by $K_{\text{a,app}}$.

3. Experimental

3.1. Instrumentation and methods

All CZE and MEKC experiments were carried out on a BioFocus 3000 Capillary Electrophoresis System (Bio-Rad, Hercules, CA, USA) at a constant temperature of 25°C. Pressure injection was carried out with an injection constant of 2 p.s.i. s, (1 p.s.i. = 6894.76 Pa) unless noted otherwise. Two fused-silica capillaries (50 μm I.D., total length 70.0 cm, distance between injection and detection 65.4 cm or 75 μm I.D., total length 50.0 cm, distance between injection and detection 45.5 cm) from Chrompack (Middelburg, Netherlands) were used. UV absorbance spectra were measured with a Perkin-Elmer UV-Vis Lambda 3B spectrophotometer (Perkin-Elmer, Cupertino, CA, USA), with 1 cm cuvettes at 25°C. Non-linear regression of Eqs. (4,5,7,13) was carried out with SlideWrite Plus (Advanced Graphics Software, Carlsbad, CA, USA), using the Levenberg-Marquardt algorithm.

3.2. Samples and solutions

Methylparaben, ethylparaben, propylparaben and butylparaben were obtained from Sigma (St. Louis, MO, USA), 2,4,5-trichlorophenol, o-nitrophenol, p-nitrophenol and polyoxyethylene(23)dodecanol (Brij 35) were obtained from Merck (Darmstadt, Germany), sodium dodecyl sulphate (SDS) was obtained from Aldrich (Steinheim, Germany). All other chemicals were of analytical-reagent grade. Samples were dissolved in water at a final concentration of 0.25 mM, unless noted otherwise. Sudan III was used as a micelle marker to determine m_{MC}. Water was purified using a Milli-Q water purification system

Table 1
Composition of electrolyte systems at different pH values

Cation[a]	Buffering counter-species[a]	pH
0.02 M Tris	Formic acid	4.0
0.02 M Tris	Acetic acid	5.0
0.02 M Tris	MES	6.0
0.02 M Tris	o-Phosphoric acid	7.0
0.02 M Tris	Acetic acid	8.0
0.02 M Tris	Boric acid	9.0
0.01 M NaOH	CAPS	10.0
0.01 M NaOH	CAPS	11.0

[a] Tris = tris(hydroxymethyl)aminomethane; MES = 2-(N-morpholino)ethanesulphonic acid; CAPS = 3-cyclohexylamino-1-propanesulfonic acid.

(Waters Millipore, Milford, MA, USA). In Table 1, the compositions of the electrolyte systems at different pH values are listed. These electrolyte systems were selected in order to ascertain a good buffering capacity and relatively low currents in the complete pH range of 4–11. It should be noted that, both in the mobility and the retention model, the influence of the surfactant in the electrolyte system on ionic strength and, consequently, on ionic mobility is not taken into account. Therefore the small differences in ionic strength between the electrolyte systems, listed in Table 1, were considered to be negligible. All buffer solutions were filtered through 0.45 μm filters prior to use.

4. Results and discussion

4.1. Migration behaviour in CZE

In order to study the migration behaviour of monovalent acids in CZE, experiments were carried out using the electrolyte systems listed in Table 1. From the measured migration times, effective mobilities, $m_{eff,AQ}$, were calculated and $m_{A^-,AQ}$ and pK_a values were determined, using Eq. (5). These results are presented in Fig. 1 and Table 2. For comparative purposes with the MEKC data, the subscript AQ is also applied in the CZE data. The calculated pK_a values are in good agreement with those reported in literature [15]. As expected from theory, a decrease in $m_{A^-,AQ}$ is observed for the alkylparabens with increasing alkyl chain length.

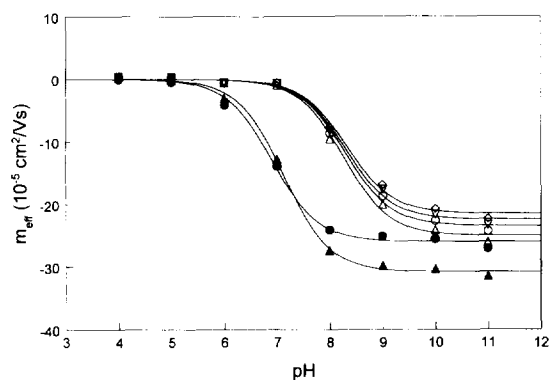Fig. 1. Effective mobility, m_{eff}, versus pH for (△) methylparaben, (○) ethylparaben, (▽) propylparaben, (◊) butylparaben, (▲) o-nitrophenol and (●) 2,4,5-trichlorophenol. Capillary, 50 μm I.D., $l_c = 70.0$ cm, $l_d = 65.4$ cm. Voltage, 20 kV. Lines represent calculated curves according to Eq. (5).

4.2. Migration behaviour in MEKC

From the results shown in Fig. 1, it can be concluded that alkylparabens can be separated by CZE at high pH. However, the differences in effective mobility are rather small. Much better separations are obtained by MEKC, due to the additional separation mechanism based on micellar solubilization. This is illustrated in Fig. 2 for an electrolyte system of 0.02 M Tris–boric acid at pH 9.0. In CZE (Fig. 2a), the migration behaviour of the alkylparabens is mainly determined by their effective hydrated radii. In MEKC (Fig. 2b), a reversal of the migration order is obtained, due to stronger interactions of the larger, more hydrophobic alkylparabens with the pseudo-stationary micellar phase.

Table 2
Effective mobility of the fully dissociated acid, $m_{A^-,AQ}$ (10^{-5} cm^2/V s), and pK_a values with standard deviations (in parentheses), $n = 8$, determined using Eq. (5) and pK_a values from literature

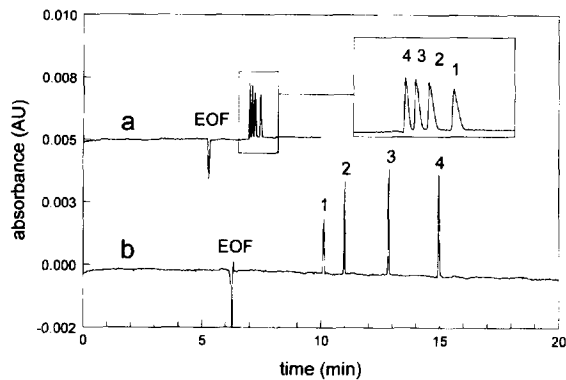| Compound	$m_{A^-,AQ}$	pK_a	pK_a lit.[a]
Methylparaben	−25.04 (0.53)	8.27 (0.06)	
Ethylparaben	−23.47 (0.48)	8.29 (0.06)	
Propylparaben	−22.43 (0.51)	8.30 (0.06)	
Butylparaben	−21.49 (0.48)	8.32 (0.06)	
o-Nitrophenol	−30.78 (0.33)	7.31 (0.03)	7.23
2,4,5-Trichlorophenol	−25.99 (0.41)	6.91 (0.05)	6.72

[a] Data from Ref. [15].

Fig. 2. Electrokinetic chromatograms for the separation of (1) methylparaben, (2) ethylparaben, (3) propylparaben and (4) butylparaben in an electrolyte system consisting of 0.02 M Tris–boric acid, pH 9.0, containing (a) 0 mM and (b) 50 mM SDS. Capillary, 50 μm I.D., $l_c = 70.0$ cm, $l_d = 65.4$ cm. Voltage, 20 kV. Detection wavelength, 200 nm.
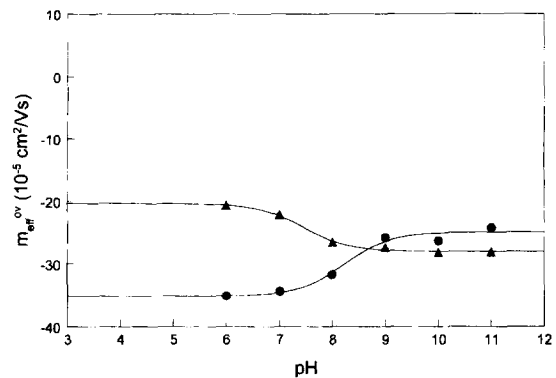

Fig. 3. Overall effective mobility, m_{eff}^{ov}, versus pH for (▲) o-nitrophenol and (●) 2,4,5-trichlorophenol. Capillary, 50 μm I.D., $l_c = 70.0$ cm, $l_d = 65.4$ cm. Voltage, 20 kV. SDS concentration, 50 mM. Lines represent calculated curves according to Eq. (7).

4.2.1. Mobility model

To study the migration behaviour of monovalent weak acids in MEKC, experiments were carried out using the electrolyte systems from Table 1 in the pH range of 6–11, containing 50 mM SDS. With the electrolyte systems at pH values of 4.0 and 5.0, the restricted elution mode was obtained where the micelle migration time could not be determined [16]. From the measured migration times, effective overall mobilities, m_{eff}^{ov}, were calculated and m_{HA}, m_{A^-} and p$K_{a,app}$ values were determined, using Eq. (7). The results are listed in Table 3. The relatively small differences between m_{A^-} and $m_{A^-,AQ}$ (see Table 2) indicate that the dissociated forms of the sample compounds do not interact with the micellar phase, due to electrostatic repulsion. The small decrease of m_{A^-} compared to $m_{A^-,AQ}$ may be attributed to an

increase in the ionic strength and viscosity of the micellar electrolyte system. It should be noted that with a decrease in mobility, we mean a decrease in the absolute value of the mobility. The low value for butylparaben is due to a less accurate fit. As expected from Eq. (8), an increase in m_{HA} is observed for the alkylparabens with an increase in hydrophobicity. The ΔpK_a values demonstrate that higher pK_a shifts are obtained for more hydrophobic species, due to a stronger interaction with the micellar phase. For methylparaben, the variation in overall effective mobility as a function of pH is small, which makes the determination of p$K_{a,app}$ more difficult. In Fig. 3, the overall effective mobility is shown as a function of pH for o-nitrophenol and 2,4,5-trichlorophenol. At low pH (undissociated form), the migration is mainly determined by micellar solubilization, whereas at high pH (dissociated

Table 3
Values of m_{HA} (10^{-5} cm^2/V s), m_{A^-} (10^{-5} cm^2/V s) and p$K_{a,app}$ with standard deviations (in parentheses), $n = 6$, determined using Eq. (7) and ΔpK_a values

Compound	m_{HA}	m_{A^-}	p$K_{a,app}$	ΔpK_a
Methylparaben	−26.58 (0.44)	−23.87 (0.33)	7.94 (0.40)	−0.33
Ethylparaben	−32.38 (0.51)	−22.44 (0.72)	9.17 (0.18)	0.88
Propylparaben	−35.77 (0.33)	−20.54 (0.79)	9.90 (0.09)	1.60
Butylparaben	−37.64 (0.35)	−17.43 (2.23)	10.57 (0.15)	2.25
o-Nitrophenol	−20.31 (0.35)	−28.01 (0.19)	7.45 (0.10)	0.14
2,4,5-Trichlorophenol	−35.11 (0.72)	−24.92 (0.62)	8.24 (0.19)	1.33

SDS concentration, 50 mM.

form), the migration is mainly determined by the effective mobility in the aqueous phase.

These results indicate that MEKC offers a possibility for controlling the migration behaviour in order to improve the separation of charged compounds with almost identical effective mobilities, as stated by Khaledi et al. [7], provided that the degree of interaction with the micellar phase is sufficiently different. In Fig. 4, the influence of a SDS pseudostationary phase on the migration of o-nitrophenol and 2,4,5-trichlorophenol is illustrated for electrolyte systems at pH values of 6.0 and 11.0, respectively. At pH 6.0 (Fig. 4a,b, strong interaction) large mobility differences are obtained, whereas at pH 11.0 (Fig. 4c,d, weak interaction) only small differences are observed.

4.2.2. Retention model

As was pointed out in Section 2, the migration behaviour of charged compounds in MEKC can also be described in terms of a retention factor [7]. For all sample compounds, retention factors, k, were calcu-
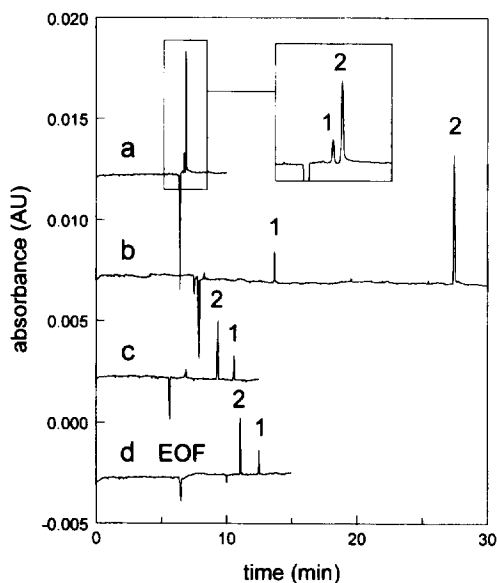

Fig. 5. Retention factor versus pH for ($\triangle$) methylparaben, ($\bigcirc$) ethylparaben, (∇) propylparaben and ($\Diamond$) butylparaben. Capillary, 50 μm I.D., $l_c = 70.0$ cm, $l_d = 65.4$ cm. Voltage, 20 kV. SDS concentration, 50 mM. Lines represent calculated curves according to Eq. (4).

lated according to Eq. (3), using the effective mobilities obtained with CZE experiments (see Fig. 1) for $m_{\mathrm{eff,AQ}}$. Sigmoidal relationships were obtained for all sample compounds, which is illustrated in Fig. 5 for four alkylparabens. From these data, k_{HA}, $k_{\mathrm{A^-}}$ and pK_a values were determined using Eq. (4). The results are presented in Table 4. From the low values for $k_{\mathrm{A^-}}$ and the corresponding standard deviations, it can be concluded that $k_{\mathrm{A^-}}$ equals zero for all compounds. These results support the suggestion that the dissociated forms of the sample compounds do not interact with the micellar phase. An increase in k_{HA} is observed with an increase in hydrophobicity. The calculated pK_a values are comparable with those obtained with CZE experiments, as listed in Table 2. A higher value was obtained for butylparaben, due to a less accurate fit.

Fig. 4. Electrokinetic chromatograms for the separation of (1) o-nitrophenol and (2) 2,4,5-trichlorophenol in electrolyte systems of (a and b) 0.02 M Tris–MES, pH 6.0, and (c and d) 0.02 M Tris–CAPS, pH 11.0, containing (a and c) 0 mM and (b and d) 50 mM SDS. Capillary, 50 μm I.D., $l_c = 70.0$ cm, $l_d = 65.4$ cm. Voltage, 20 kV. Detection wavelength, 200 nm.
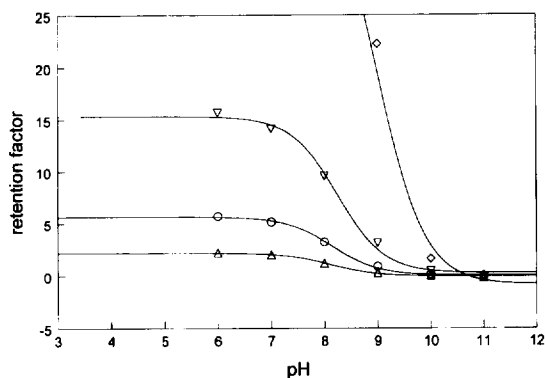
Table 4
Values of k_{HA}, $k_{\mathrm{A^-}}$ and pK_a with standard deviations (in parentheses), $n = 6$, determined using Eq. (4)

Compound	k_{HA}	$k_{\mathrm{A^-}}$	pK_a
Methylparaben	2.19 (0.07)	−0.07 (0.06)	8.12 (0.08)
Ethylparaben	5.64 (0.14)	0.01 (0.11)	8.15 (0.06)
Propylparaben	15.32 (0.44)	0.24 (0.38)	8.24 (0.08)
Butylparaben	39.54 (3.41)	−0.84 (4.45)	9.01 (0.27)
o-Nitrophenol	1.13 (0.07)	−0.24 (0.03)	7.14 (0.10)
2,4,5-Trichlorophenol	14.60 (0.70)	−0.08 (0.17)	6.64 (0.07)

SDS concentration, 50 mM.

4.3. Mobility model versus retention model

It can be concluded that both the mobility model and the retention model describe the migration behaviour of monovalent weak acids during MEKC analyses well. For the correct calculation of retention factors according to Eq. (3), effective mobilities in the aqueous phase are required. However, $m_{eff,AQ}$ is a difficult quantity to measure, which makes the determination of retention factors troublesome. This difficulty has also been addressed by others [6,17]. As mentioned in Section 2, several assumptions are made if mobility data from CZE experiments are used for retention factor calculations. A fundamental difference between the composition of the aqueous phase in MEKC and the electrolyte system in CZE is the presence of surfactant monomer at a concentration equal to the critical micelle concentration (CMC).

In order to study the influence of the surfactant concentration on $m_{eff,AQ}$, experiments were carried out in electrolyte systems with pH values of 7.0 and 9.0, containing different SDS concentrations, including concentrations below the CMC. These pH values were selected to ensure that partial dissociation of the sample compounds occurred. In Fig. 6, the experimental overall effective mobilities are shown

for (A) o-nitrophenol and 2,4,5-trichlorophenol at pH 7.0 and (B) four alkylparabens at pH 9.0. In these figures, the CMCs are indicated also; these were determined from the inflection points of the measured current versus the concentration of SDS. Above the CMC, an increase in m_{eff}^{ov} is obtained for all sample compounds, due to micellar solubilization. Below the CMC, a small decrease is observed in $m_{eff,AQ}$ for o-nitrophenol, due to an increase in the ionic strength. For 2,4,5-trichlorophenol, however, a large increase in $m_{eff,AQ}$ is obtained below the CMC, with an increase in the concentration of SDS (see Fig. 6A). These experiments were repeated three times with freshly prepared electrolyte systems and sample solutions and identical results were obtained in each case. The same behaviour was observed, although to a lesser extent, for propylparaben and butylparaben (see Fig. 6B). We assume that this phenomenon is due to interaction of the undissociated forms of the sample compounds with surfactant monomers. Several authors have reported the separation of neutral compounds based on hydrophobic interaction with surfactant monomers [18–20]. This type of interaction probably occurs also in the aqueous phase during MEKC experiments, resulting in an increase of $m_{eff,AQ}$ for hydrophobic compounds. To investigate the influence of the
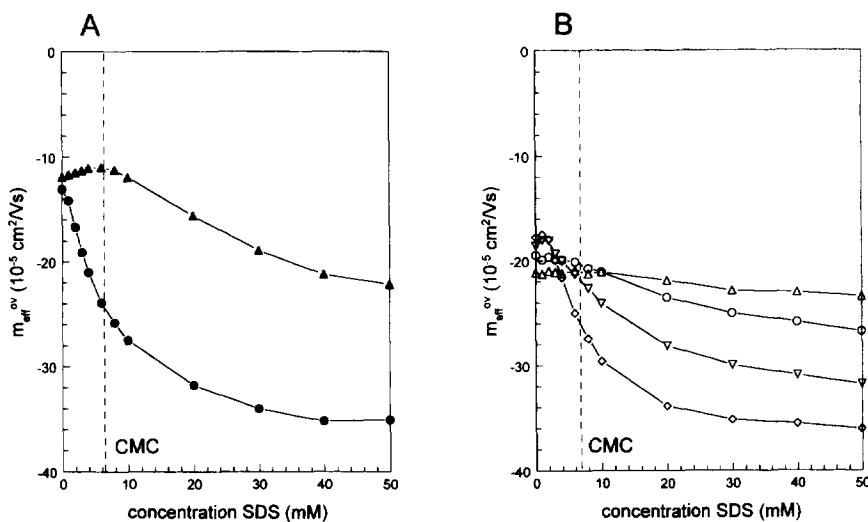

Fig. 6. Overall effective mobility, m_{eff}^{ov}, as a function of the concentration of SDS for (△) methylparaben, (○) ethylparaben, (▽) propylparaben, (◇) butylparaben, (▲) o-nitrophenol and (●) 2,4,5-trichlorophenol in electrolyte systems of (A) 0.02 M Tris–o-phosphoric acid at pH 7.0 and (B) 0.02 M Tris–boric acid at pH 9.0. Capillary, 50 μm I.D., $l_c = 70.0$ cm, $l_d = 65.4$ cm. Voltage, 20 kV.

degree of dissociation on the interaction with surfactant monomers in more detail, additional experiments were carried out in electrolyte systems at pH values of 5.0 and 9.0, respectively. A decrease in interaction was observed for 2,4,5-trichlorophenol with an increase in the degree of ionization (i.e., at higher pH), as shown in Fig. 7. These results support the suggestion that the undissociated forms of hydrophobic compounds may show interaction with surfactant monomers. Vindevogel and Sandra [6] reported different results for charged species in borate and Tris buffers, indicating that the chemical nature of the buffer may also affect the interaction mechanism.

In Table 5, values of $m_{eff,AQ}$ are listed for all sample compounds for a CZE electrolyte system (surfactant concentration, $C_{sf}=0$) and for a MEKC electrolyte system ($C_{sf}=$ CMC), respectively. Large differences are observed for hydrophobic compounds

Table 5
Effective mobility in the aqueous phase, $m_{eff,AQ}$ (10^{-5} cm^2/V s), determined from Fig. 6, at SDS concentrations $C_{sf}=0$ and CMC, respectively

Compound	$m_{eff,AQ}$ ($C_{sf}=0$)	$m_{eff,AQ}$ ($C_{sf}=$ CMC)
Methylparaben	−21.14	−21.05
Ethylparaben	−19.47	−20.36
Propylparaben	−18.58	−21.85
Butylparaben	−17.75	−26.01
o-Nitrophenol	−11.93	−11.06
2,4,5-Trichlorophenol	−13.09	−24.45

Electrolyte systems, 0.02 M Tris–boric acid at pH 9.0 (alkylparabens) and 0.02 M Tris–o-phosphoric acid at pH 7.0 (o-nitrophenol and 2,4,5-trichlorophenol).

and these will have a pronounced effect on the calculation of retention factors according to Eq. (3). This is illustrated in Fig. 8 for o-nitrophenol and 2,4,5-trichlorophenol, where k is shown as a function
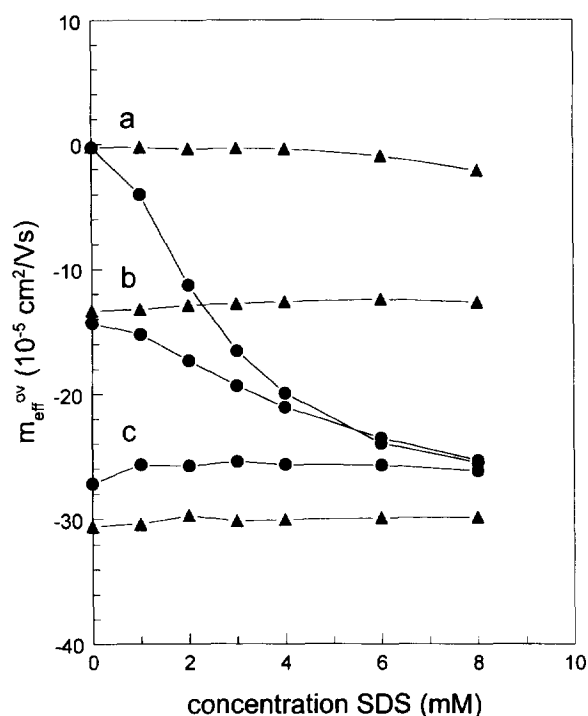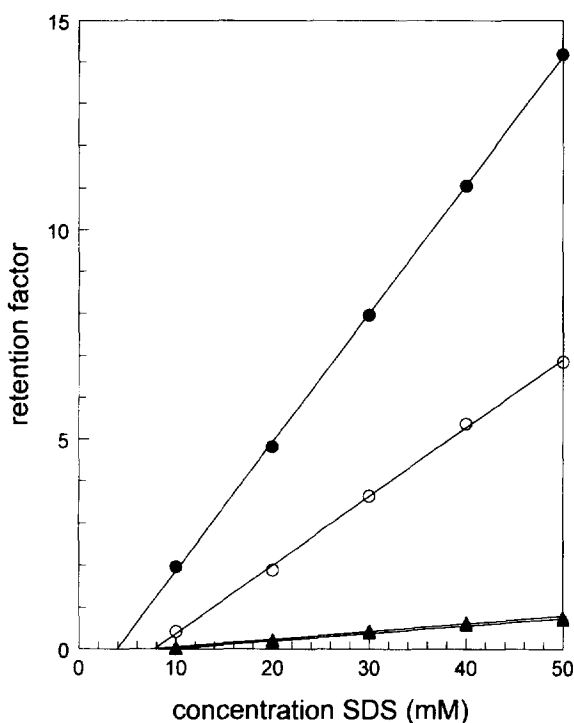

Fig. 7. Overall effective mobility, m_{eff}^{ov}, as a function of the concentration of SDS for (▲) o-nitrophenol and (●) 2,4,5-trichlorophenol in electrolyte systems of (a) 0.02 M Tris–acetic acid at pH 5.0, (b) 0.02 M Tris–o-phosphoric acid at pH 7.0 and (c) 0.02 M Tris–boric acid at pH 9.0. Capillary, 50 μm I.D., $l_c=70.0$ cm, $l_d=65.4$ cm. Voltage, 20 kV.

Fig. 8. Calculated retention factor using Eq. (3) versus the concentration of SDS for (▲) o-nitrophenol and (●) 2,4,5-trichlorophenol. Calculations with (closed symbols) $m_{eff,AQ}$ at $C_{sf}=0$ and (open symbols) $m_{eff,AQ}$ at $C_{sf}=$ CMC, respectively. Electrolyte system, 0.02 M Tris–o-phosphoric acid at pH 7.0. Capillary, 50 μm I.D., $l_c=70.0$ cm, $l_d=65.4$ cm. Voltage, 20 kV.

of the concentration of SDS. Although linear graphs are obtained with both $m_{eff,AQ}$ values, large differences in k are observed for 2,4,5-trichlorophenol.

These results demonstrate that mobility data obtained with CZE experiments should be used with caution to calculate retention factors of charged compounds in MEKC experiments. Therefore, the mobility model is to be preferred to describe their migration behaviour. Moreover, overall effective mobilities are more directly related to migration times in the electrokinetic chromatogram and give a better indication of whether sample compounds can be separated or not. These findings have also been reported by others [7].

4.4. Spectroscopic pK_a determination

Due to partitioning with the micellar pseudo-stationary phase, shifts in dissociation constants of weak acids can be observed in MEKC experiments [7,21]. The magnitude of these pK_a shifts depends on the structural properties of the solute and the micelles, and, as demonstrated in Table 3, can be more than two pH units in a SDS micellar system. The migration models described above demonstrate that dissociation constants play an important role in the migration behaviour. Therefore, a good determination of pK_a values in micellar media will be beneficial for a better understanding of the separation mechanism and for developing optimization strategies. Moreover, dissociation constants play a key role in several chemical and biological phenomena, such as reaction kinetics and biological uptake and transport. Since most capillary electrophoresis instruments are equipped with a UV detector, this technique enables the simultaneous determination of pK_a by electrophoretic and spectroscopic methods. To investigate this possibility, CZE and MEKC experi-

ments were carried out with p-nitrophenol in electrolyte systems consisting of 0.02 M Tris–acetate in the pH range of 5–10, with steps of one pH unit. For the MEKC experiments, 50 mM SDS and 50 mM Brij 35 (neutral surfactant) were applied as the pseudo-stationary phases. For the spectrophotometric method, the best results are obtained when the difference between the molar absorptivities of the undissociated form, ϵ_{HA}, and the dissociated form, ϵ_{A^-}, are large. In order to select proper wavelengths, the UV absorbance spectra of p-nitrophenol were measured in an aqueous solution, a 50 mM SDS solution and a 50 mM Brij 35 solution, containing 0.01 M NaOH or 0.01 M HCl, respectively. The local maxima of the undissociated form ($\lambda = 317$ nm) and the dissociated form ($\lambda = 407$ nm) were found to be unaffected by the presence of the surfactant. These wavelengths were used for detection. From the measured migration times, overall effective mobilities were calculated and dissociation constants were determined using Eq. (7). From the measured temporal peak areas, spatial peak areas were calculated and dissociation constants were determined using Eq. (13). The results are presented in Table 6, showing that 50 mM Brij 35 causes a larger pK_a shift for p-nitrophenol than does 50 mM SDS. Considerable differences are observed between the two methods for the 50 mM SDS electrolyte system. These differences may be attributed to a limited buffering capacity of the applied electrolyte system at some pH values, difficulties in integrating small peaks and the relatively large steps of one pH unit. Better results were reported for CZE experiments by Cleveland et al. [14], with an equation including activity correction for buffer ionic strength, applying more migration data obtained with different buffers and UV detection at 237 nm. Comparing the results with those of o-nitrophenol, as listed in Tables 2 and 3, we believe that the dissociation constants of p-nitro-

Table 6

Dissociation constants of p-nitrophenol, determined with mobility data, m_{eff}^{ov}, according to Eq. (7) and spatial peak areas, A_s, at 317 and 407 nm using Eq. (13) with standard deviations (in parentheses), $n = 6$, and ΔpK_a values

	CZE	50 mM SDS	ΔpK_a	50 mM Brij 35	ΔpK_a
m_{eff}^{ov}	7.29 (0.11)	7.40 (0.12)	0.11	7.97 (0.06)	0.68
A_s^{317}	7.27 (0.12)	7.66 (0.18)	0.39	7.94 (0.08)	0.67
A_s^{407}	7.44 (0.17)	7.96 (0.10)	0.52	7.96 (0.01)	0.52

phenol determined with m_{eff}^{ov} are the most reliable values.

4.5. m_{eff}^{ov}-mediated sample stacking

Differences in overall effective mobilities caused by a neutral surfactant can be used for a sample stacking procedure of weak acids. This procedure is schematically illustrated in Fig. 9 and works as follows: The acid is dissolved in an electrolyte system at a pH that is close to its pK_a value. The analysis is carried out with the same electrolyte system, containing a neutral surfactant. The presence of the neutral surfactant will lead to a decrease in the overall effective mobility of the acid, due to (i) a micelle-induced pK_a shift resulting in a lower degree of dissociation, (ii) partitioning with neutral micelles and (iii) an increase in the viscosity of the electrolyte system. The higher effective mobility in the sample solution will result in a higher linear velocity and consequently in stacking of the acid at the rear side of the sample plug. In this way, sample stacking is possible, even if the sample is dissolved in a solution with the same ionic strength as that of the electrolyte system. This procedure was demonstrated for p-nitrophenol, using an electrolyte system of 0.02 M
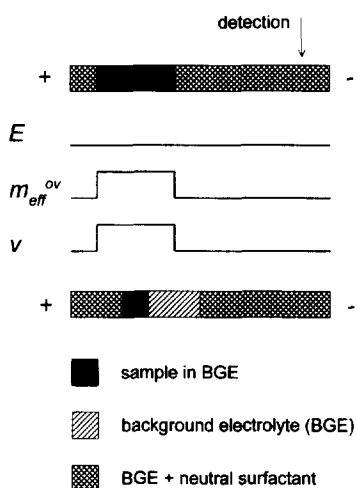
Tris–MOPS (morpholinopropane sulphonic acid) at pH 7.0 and 50 mM Brij 35 as the neutral micellar phase. For comparative purposes, a field-amplified sample stacking procedure was also carried out, i.e., sample was dissolved in deionized water [22]. Notice that in this situation the higher linear velocity in the sample plug is based on a higher field strength and not on a higher effective mobility. In Fig. 10, the results are shown for (a) no sample stacking, (b) field-amplified sample stacking and (c) m_{eff}^{ov}-mediated sample stacking, using injection constants of 2 and 20 p.s.i. s, respectively. Without sample stacking, a broad sample zone is obtained for the long injection time. The gradual shape at the front side of the plug is due to the parabolic flow profile during hydrodynamic injection. The migration times for the two injection constants (front side of the peak) are almost identical. With field-amplified sample stacking, good peak shapes are obtained for both injection times. Due to differences in the electroosmotic mobilities of water and the electrolyte system, a higher EOF is
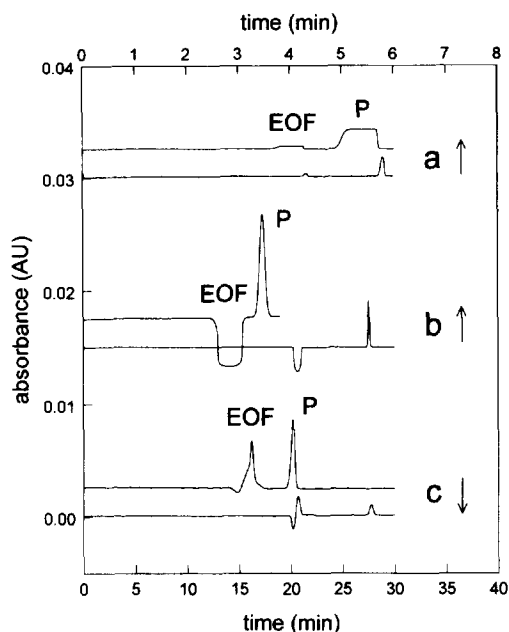

Fig. 9. Schematic representation of m_{eff}^{ov}-mediated sample stacking. Sample was dissolved in background electrolyte and the capillary was filled with background electrolyte containing a neutral surfactant. E = field strength, m_{eff}^{ov} = overall effective mobility and v = linear velocity. For further explanation, see text.

Fig. 10. Electrokinetic chromatograms for p-nitrophenol dissolved in (a and c) background electrolyte and (b) water. Electrolyte system, 0.02 M Tris–MOPS at pH 7.0 containing (a and b) 0 mM and (c) 50 mM Brij 35. Injection constant, (upper curve) 20 and (lower curve) 2 p.s.i. s. Capillary, 75 μm I.D., l_c = 50.0 cm, l_d = 45.4 cm. Voltage, 15 kV. Detection wavelength, 215 nm.

obtained at longer injection times. For the long injection constant, it takes more time for the sample compound to migrate out of the sample plug and, therefore, it is detected closer to the EOF. With m_{eff}^{ov}-mediated sample stacking, good peak shapes are also obtained for both injection times. The injected amount is smaller and the migration times are longer, due to the higher viscosity of the electrolyte system. For the longer injection time, a higher EOF is obtained, due to the higher electroosmotic mobility of the sample plug. Here, it takes more time for the sample compound to migrate out of the long sample plug. In order to investigate the contribution of the increase in viscosity to the sample stacking procedure, the viscosity ratio of electrolyte systems with and without 50 mM Brij 35 was determined. A solution of the electrolyte system, containing 1 μl/ml mesityl oxide, was injected for a long period and the time that it took to reach the detector was measured. The ratio of the times for both electrolyte systems equals their viscosity ratio, which was 1.76. The ratio of overall effective mobilities of p-nitrophenol in these electrolyte systems was 5.91, illustrating that the contribution of differences in viscosity is small and that the decrease in m_{eff}^{ov} is based mainly on micellar partitioning. In Fig. 11, peak heights are shown as a function of the injection constant. Without sample stacking, the maximum peak height is obtained with 2 p.s.i. s. With field-

amplified sample stacking, peak shapes start to deteriorate with longer sample plugs, due to a mismatch in the EOF of the sample plug and that of the electrolyte system [23]. For the m_{eff}^{ov}-mediated sample stacking procedure, the curve starts to level off at higher injection constants, which is due also to an EOF mismatch.

5. Conclusions

Both the retention model and the mobility model can be applied to describe the migration behaviour of monovalent weak acids during MEKC experiments. However, the determination of effective mobilities in the aqueous phase may be troublesome, due to interaction of the undissociated form of the acid with surfactant monomers. This phenomenon may have a marked influence on the calculation of retention factors for hydrophobic species. The results demonstrate that retention factors, calculated with mobility data obtained with CZE experiments, should be used cautiously. Moreover, overall effective mobilities are more directly related to the migration behaviour than to retention factors. Therefore, the mobility model is preferable for describing the migration behaviour of charged compounds in MEKC.

Micelle-induced pK_a shifts may occur during MEKC analyses, due to micellar solubilization. CZE and MEKC were shown to be suitable techniques for determining dissociation constants in aqueous and micellar media by electrophoretic and spectroscopic methods simultaneously. It was demonstrated that differences in overall effective mobilities, based on interaction with a neutral surfactant, can be applied for a sample stacking procedure, even if the sample compound is dissolved in a solution with the same ionic strength as that of the applied electrolyte system.
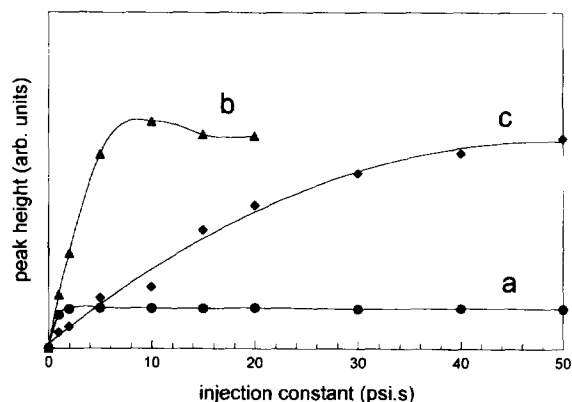

Fig. 11. Peak height versus injected amount under (a) non-stacking, (b) field-amplified sample stacking and (c) m_{eff}^{ov}-mediated sample stacking conditions. Capillary, 75 μm I.D., l_c = 50.0 cm, l_d = 45.4 cm. Voltage, 15 kV. Detection wavelength, 215 nm.

Acknowledgments

References

[1] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, Anal. Chem., 56 (1984) 111.

[2] S. Terabe, K. Otsuka and T. Ando, Anal. Chem., 57 (1985) 251.

[3] H. Nishi, N. Tsumagari, T. Kakimoto and S. Terabe, J. Chromatogr., 477 (1989) 259.

[4] H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, J. Chromatogr., 498 (1990) 313.

[5] H. Nishi, N. Tsumagari and S. Terabe, Anal. Chem., 61 (1989) 2434.

[6] J. Vindevogel and P. Sandra, J. High Resolut. Chromatogr., 14 (1991) 795.

[7] M.G. Khaledi, S.C. Smith and J.K. Strasters, Anal. Chem., 63 (1991) 1820.

[8] J.K. Strasters and M.G. Khaledi, Anal. Chem., 63 (1991) 2503.

[9] S.C. Smith and M.G. Khaledi, J. Chromatogr., 632 (1993) 177.

[10] C. Quang, J.K. Strasters and M.G. Khaledi, Anal. Chem., 66 (1994) 1646.

[11] K.L. Rundlett and D.W. Armstong, J. Chromatogr. A, 721 (1996) 173.

[12] I.V. Berezin, K. Martinek and A.K. Yatsiminski, Russ. Chem. Rev., 42 (1973) 778.

[13] X. Huang, W.F. Coleman and R.M. Zare, J. Chromatogr., 480 (1989) 95.

[14] J.A. Cleveland, C.L. Martin and S.J. Gluck, J. Chromatogr. A, 679 (1994) 167.

[15] T. Hirokawa, M. Nishino, N. Oaki, Y. Kiso, Y. Sawamoto, T. Yagi and J. Akiyama, J. Chromatogr., 271 (1983) D1–D106.

[16] P.G. Muijselaar, H.A. Claessens and C.A. Cramers, J. Chromatogr. A, 696 (1995) 273.

[17] K. Otsuka, S. Terabe and T. Ando, J. Chromatogr., 348 (1985) 39.

[18] M.M. Bushey and J.W. Jorgenson, Anal. Chem., 61 (1989) 491.

[19] E.S. Ahuja and J.P. Foley, J. Chromatogr. A, 680 (1994) 73.

[20] P.G. Muijselaar, H.B. Verhelst, H.A. Claessens and C.A. Cramers, J. Chromatogr. A, 764 (1997) 27.

[21] M.G. Khaledi and A.H. Rodgers, Anal. Chim. Acta, 239 (1990) 121.

[22] D.S. Burgi and R.L. Chien, Anal. Chem., 63 (1991) 2042.

[23] J.L. Beckers and M.T. Ackermans, J. Chromatogr., 629 (1993) 371.