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Interpretation of migration behaviour of inorganic cations in capillary ion electrophoresis based on an equilibrium model

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Abstract

In capillary ion electrophoresis, the migration of inorganic cations in an aqueous–organic medium is influenced by complexation and interactions between the solutes and organic solvents. This can be accounted for by multiple chemical equilibria. Based on these equilibria, a migration model is proposed that describes the electrophoretic mobility of the cations in terms of absolute mobility, complex formation constants and the concentrations of complexing reagents and organic solvent. It was validated in a background electrolyte composed of imidazole, 2-hydroxyisobutyric acid (HIBA), 18-crown-6 and methanol. The parameters in this model were estimated experimentally and were in good agreement with the values in the literature. The model is useful for predicting the mobilities as a function of the concentrations of HIBA, 18-crown-6 and methanol, and consequently facilitates selectivity optimization.

1. Introduction

Capillary ion electrophoresis (CIE) has been widely studied for the determination of low-molecular-mass ions [1–4]. For inorganic ions, which generally have no optical absorption, indirect on-column detection is mostly applied [5]. By introducing an absorbing substance, so-called co-ion, into the electrolyte buffer, a constant background absorbance is obtained. The zones of non-absorbing ionic species are monitored because they lead to a decrease in light absorbance. The ideal co-ion should have a similar electrophoretic mobility to the analyte ions and have a higher absorption at a given detection wavelength to achieve the highest separation efficiency and detection sensitivity

[6,7]. Imidazole, which has been found to be a suitable co-ion for the separation of alkali, alkaline earth and transition metal cations by CIE [8–10], was used in this work.

Separation in capillary electrophoresis is based on differences in electrophoretic mobilities. Ions with identical charge and size have identical electrophoretic mobilities, for instance, transition and rare earth metal cations, and the electrophoretic separation of these ions as such is impossible. However, their mobilities can be modified by introducing a chemical equilibrium in which the ions participate to form complexes. If the ions have different complex formation constants, their apparent electrophoretic mobilities should differ. The electrophoretic mobilities can also be affected by pH when the complexing reagent is a weak acid [11]. A second complexing reagent, such as a cyclic polyether, can be

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applied if the simultaneous separation of all of the cations in question is not achieved. In addition, it has been found that the volume fraction of organic solvents in water can alter the magnitude of electrophoretic mobilities and consequently modify the separation selectivity [10, 12].

Up to now, the quantitative description of the electrophoretic mobilities of inorganic cations in capillary zone electrophoresis (CZE) has been little studied. Hirokawa et al. [13] studied the isotachophoretic electrophoresis separation of fourteen rare earth metal cations (Ln) using three different electrolyte systems, acetate acid (Ac), 2-hydroxyisobutyric acid (HIBA) and a mixture of Ac and HIBA. On the basis of multiple complex formation equilibria, they determined the absolute mobilities of Ln–Ac, Ln–HIBA and Ln–Ac–HIBA complexes and the formation constants of Ln–HIBA–Ac complexes from experimental data. It was concluded that the separation was due to the differences in the effective mobilities of the Ln cations caused by the different abundances of the complexes. Using a similar principle, Swaile and Sepaniak [14] described the electrophoretic mobilities of Ca^{2+} and Mg^{2+} in CZE as a function of the concentration of 8-hydroxyquinoline-5-sulfonic acid (HQS) and pH. Employing literature values for the formation constants of metal–HQS complexes and the acid dissociation constant of HQS, they calculated the mobilities of free Ca^{2+} and Mg^{2+} and their HQS complexes from experimental data. Quang and Khaledi [15] tried to describe the mobilities of metal cations as a function of the concentration of HIBA and pH using a phenomenological expression, but did not succeed because there were too many parameters involved in the expression. They were successful in doing so using an empirical expression. In the course of the preparation of this paper, we learned that Vogt and Conradi [16] have used a theoretical model to describe the mobility of rare earth metal cations with HIBA, lactate and acetate complexations. In their model, the effects of pH and ligand are considered simultaneously.

In a recent report Sahota and Khaledi [17],

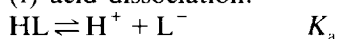
stressed the advantages of non-aqueous electrophoresis. They observed a lower current in a formamide medium. This allows an increase in ionic strength of the electrolyte buffer, and the application of a higher electric field and larger sample load in order to have a net positive effect on detection, efficiency and analysis time.

In this work, the migration behaviour of inorganic cations was studied in an organic–aqueous medium (methanol–water) with HIBA and 18-crown-6 ether as complexing agents. Based on multiple chemical equilibria, a migration model was developed, which was used to optimize the separation of twelve inorganic cations (NH_4^+ , Li^+ , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Mn^{2+} , Ni^{2+} , Zn^{2+} and Cu^{2+}).

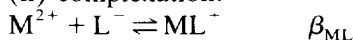
2. Theory

In CIE, when an electrolyte buffer is composed of a complexing reagent HL (a weak acid), a cyclic polyether C and an organic solvent S, the following chemical equilibria take place:

(i) acid dissociation:



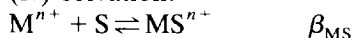
(ii) complexation:



(iii) inclusion complexation:



(iv) solvation:



where K_a , β_{ML} , β_{ML_2} , β_{ML_3} , β_{MC} and β_{MS} represent the equilibrium constants.

In (i), a weak-acid complexing reagent undergoes the acid dissociation in the electrolyte buffer.

In (ii), the divalent cations in question are complexed by HIBA. Mn^{2+} , Ni^{2+} and Cu^{2+} are known to form 1:1, 1:2 and 1:3 complexes, whereas the others form 1:1 and 1:2 complexes [18].

In (iii), a cyclic polyether C (i.e., 18-crown-6 and 15-crown-5 [19,20]) can host certain cations in its cavity by forming ion–dipole bonds with its

oxygen atoms carrying lone electron pairs [21]. The magnitude of the interaction depends on the match between the ionic radius of the cation M and that of the cavity. K^+ , Sr^{2+} and Ba^{2+} are known to form 1:1 complexes with 18-crown-6 [21,22].

In (iv), an ion interacts with a group of polar solvent molecules through ion–dipole bonds and a solvation shell may be formed around the central ion [21]. For a given cation, the number of solvent molecules within the group is assumed to be the same and not to alter with the solvent concentration, which is of course an approximation. In principle, the solvent solvates all kinds of ions in solution, some to a greater extent than others, depending on the specific properties of the central ion regarding a certain solvent. However, solvation of the negatively charged complex ions has no direct influence on the mobilities of the cations under study. Moreover, as the free cations are smaller and have a higher electrical charge and density than the solvated ones, the solvation of the bare cations should have the most significant impact on the overall mobility of the cations. In light of the above considerations, only the solvation of the free cations is taken into account.

As a consequence of the simultaneous equilibria, the cations are present as the free cations and as various complexes in the electrolyte buffer. The concentrations of the various complexed species can be obtained by the following equations:

$$[ML^+] = \beta_{ML}[M][L^-] \quad (1)$$

$$[ML_2^0] = \beta_{ML_2}[M][L^-]^2 \quad (2)$$

$$[ML_3^-] = \beta_{ML_3}[M][L^-]^3 \quad (3)$$

$$[(MC)^{n+}] = \beta_{MC}[M][C] \quad (4)$$

$$[MS^{n+}] = \beta_{MS}[M][S] \quad (5)$$

L^- is the conjugated base of HL and its concentration depends on the pH of the electrolyte buffer:

$$[L^-] = \alpha_{L^-}[HL] = \frac{K_a}{[H^+] + K_a} [HL] \quad (6)$$

where α_{L^-} is the molar fraction of the complexing reagent capable of complexing with cation M and $[HL]$ the concentration of the complexing reagent in the background electrolyte.

The total cation concentration of each of the analyte cations, C_M , is the sum of various chemical species present in the electrolyte buffer:

$$\begin{aligned} C_M &= [M] + [ML^+] + [ML_2^0] + [ML_3^-] + [MS^{n+}] \\ &\quad + [MC^{n+}] \\ &= [M] + \beta_{ML}\alpha_{L^-}[HL][M] \\ &\quad + \beta_{ML_2}(\alpha_{L^-}[HL])^2[M] \\ &\quad + \beta_{ML_3}(\alpha_{L^-}[HL])^3[M] + \beta_{MC}[C][M] \\ &\quad + \beta_{MS}[S][M] \end{aligned} \quad (7)$$

Combining Eqs. 1, 2, 3, 4, 5 and 7, we obtain the expressions of molar fraction of the various ionic species α_M , α_{ML^+} , $\alpha_{ML_2^0}$, $\alpha_{ML_3^-}$ and $\alpha_{MC^{n+}}$:

$$\alpha_M = \frac{[M]}{C_M} = \frac{1}{(1 + \beta_{ML}\alpha_{L^-}[HL] + \beta_{ML_2}(\alpha_{L^-}[HL])^2 + \beta_{ML_3}(\alpha_{L^-}[HL])^3 + \beta_{MC}[C] + \beta_{MS}[S])} \quad (8)$$

$$\alpha_{ML^+} = \frac{[ML^+]}{C_M} = \frac{\beta_{ML}\alpha_{L^-}[HL]}{1 + \beta_{ML}\alpha_{L^-}[HL] + \beta_{ML_2}(\alpha_{L^-}[HL])^2 + \beta_{ML_3}(\alpha_{L^-}[HL])^3 + \beta_{MC}[C] + \beta_{MS}[S]} \quad (9)$$

$$\alpha_{ML_3^-} = \frac{[ML_3^-]}{C_M} = \frac{\beta_{ML_3}(\alpha_{L^-}[HL])^3}{1 + \beta_{ML}\alpha_{L^-}[HL] + \beta_{ML_2}(\alpha_{L^-}[HL])^2 + \beta_{ML_3}(\alpha_{L^-}[HL])^3 + \beta_{MC}[C] + \beta_{MS}[S]} \quad (10)$$

$$\alpha_{MS^{n+}} = \frac{[MS^{n+}]}{C_M} = \frac{\beta_{MS}[S]}{1 + \beta_{ML}\alpha_{L^-}[HL] + \beta_{ML_2}(\alpha_{L^-}[HL])^2 + \beta_{ML_3}(\alpha_{L^-}[HL])^3 + \beta_{MC}[C] + \beta_{MS}[S]} \quad (11)$$

$$\alpha_{MC}^{n+} = \frac{[(MC)^{n+}]}{C_M} = \frac{\beta_{MC}[C]}{1 + \beta_{ML}\alpha_L[HL] + \beta_{ML_2}(\alpha_L[HL])^2 + \beta_{ML_3}(\alpha_L[HL])^3 + \beta_{MC}[C] + \beta_{MS}[S]} \quad (12)$$

The effective electrophoretic mobility, μ_{eff} , of each resulting CZE zone is equal to the sum of the products of the mobilities of the free metal cations and the various ionic species:

$$\mu_{eff} = \mu_0\alpha_M + \mu_{ML}\alpha_{ML} + \mu_{ML_2}\alpha_{ML_2} + \mu_{ML_3}\alpha_{ML_3} + \mu_{MS}\alpha_{MS} + \mu_{MC}\alpha_{MC} \quad (13)$$

The mobility of ML_2^0 is equal to zero and is therefore not taken into account in Eq. 13. Combining Eqs. 8–13, we obtain:

$$\mu_{eff} = \frac{\mu_0 + \mu_{ML}\beta_{ML}\alpha_L[HL] + \mu_{ML_2}\beta_{ML_2}(\alpha_L[HL])^2 + \mu_{ML_3}\beta_{ML_3}(\alpha_L[HL])^3 + \mu_{MC}\beta_{MC}[C] + \mu_{MS}\beta_{MS}[S]}{1 + \beta_{ML}\alpha_L[HL] + \beta_{ML_2}(\alpha_L[HL])^2 + \beta_{ML_3}(\alpha_L[HL])^3 + \beta_{MC}[C] + \beta_{MS}[S]} \quad (14)$$

where μ_0 , μ_{ML} , μ_{ML_2} , μ_{ML_3} , μ_{MS} and μ_{MC} are the electrophoretic mobilities of the various cationic species.

The electrophoretic mobilities of the cations depend on both solute-specific parameters and condition-dependent parameters. The former are β_{ML} , β_{ML_2} , β_{ML_3} , β_{MC} , β_{MS} , μ_0 , μ_{ML} , μ_{ML_2} , μ_{ML_3} , μ_{MS} and μ_{MC} and the latter are the concentrations of complexing reagents [HL] and [C], the fraction of solvent in electrolyte buffer [S] and the pH of the buffer (see Eq. 6).

3. Experimental

3.1. Instrumentation

The CE instrument was a Waters Quanta 4000 capillary electrophoresis system with a twenty sample carousel and a zinc lamp detector (214 nm). Accusep fused-silica capillaries (60 cm × 75

μm I.D.) were used in all analyses. A positive voltage of 20 kV was applied. The detector time constant was 0.3 s. Samples were introduced by either hydrostatic or electromigration injection. The electropherograms were recorded and treated with a Waters Model 810 data workstation equipped with a W51-watch-dog interface. The temperature was kept at 23°C.

3.2. Capillary preparation and cleaning

Every morning, the capillary was washed for 1 min with 0.5 mol/l KOH, for 2 min with water purified with a Milli-Q system (Millipore) and for 3 min with the electrolyte buffer. Subsequently, it was conditioned for at least 15 min. Between each injection, the capillary was washed for 1 min with 0.1 mol/l KOH, for 1 min with Milli-Q-purified water and for 3 min with the electrolyte buffer. At the end of the day, the capillary was rinsed with Milli-Q-purified water for 5 min and left in the water.

3.3. Reagents and standards

Titrisol concentrates of 1000 μg/ml of Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Mn^{2+} , Ba^{2+} , Sr^{2+} , Cr^{3+} , Zn^{2+} and Cu^{2+} (Merck, Darmstadt, Germany) were used. Stock standard solutions containing 1000 μg/ml of Ni^{2+} , NH_4^+ and Li^+ were prepared from their chloride salts (Merck). Working standard solutions containing different concentrations of the above elements were prepared by mixing the appropriate amounts of the stock standard solutions.

Imidazole (99%, w/w) was of analytical-reagent grade (Merck) and methanol was of chromatography grade (Merck). HCl was a commercial 0.1 mol/l solution (Merck). 2-Hydroxyisobutyric acid (99%, w/w) and 18-crown-6 (99%, w/w) were of analytical-reagent grade (Aldrich-Chemie, Steinheim, Germany).

3.4. Preparation of background electrolyte

First, three stock standard solutions containing 130.6 mmol/l of HIBA, 50.0 mmol/l of 18-crown-6 and 500.0 mmol/l of imidazole were

prepared. The background electrolytes were prepared by mixing appropriate amounts of the above stock standard solutions and corresponding volumes of methanol in a 100-ml plastic volumetric flask. The pH was then adjusted to ca. 4.5 (with a pH meter) by titration with 1 mol/l HCl or KOH. Just before use they were filtered through a 0.45- μm syringe filter (Millipore, Molsheim, France).

3.5. Calculation

The electrophoretic mobility of each cation, μ_{eff} , is calculated from the following equation:

$$\mu_{\text{eff}} = \frac{L_t L_d}{V} \cdot \left(\frac{1}{t} - \frac{1}{t_0} \right) \quad (15)$$

where L_t (60 cm) is the total length of the capillary, L_d (52 cm) the distance from the injector to the detector, V the applied voltage (20 kV), t the migration time of the analyte cation and t_0 the migration time of water, which is used as the marker of electroosmotic flow (EOF).

The parameters in Eqs. 18–20 (see later) were estimated by non-linear regression using the Statistical Package for the Social Sciences (SPSS) [23]. The Levenberg–Marquardt algorithm was employed. Iteration was stopped when the relative decrease between successive residual sums of squares was $\leq 1.0 \times 10^{-8}$.

A computer program written with Matlab for Windows [24] is used for selectivity optimization.

4. Results and discussion

4.1. Effect of HIBA on migration

The effect of HIBA was studied by changing the concentration of HIBA from 0 to 10 mmol/l and in the absence of methanol and 18-crown-6 (pH 4.5). It was found that the mobilities of K^+ , Na^+ , Li^+ and NH_4^+ do not alter with increase in the concentration of HIBA (the results are not shown here), indicating that there is no complexation between these cations and HIBA. As

shown in Fig. 1a, the mobilities of Sr^{2+} , Ba^{2+} , Mg^{2+} , Ca^{2+} and Mn^{2+} decrease slightly, whereas those of Ni^{2+} , Zn^{2+} and Cu^{2+} decrease rapidly as the concentration of HIBA increases. The steepness of the decline depends on the magnitude of the formation constants and Ni^{2+} , Zn^{2+} and Cu^{2+} are known to have larger formation constants with HIBA (see Table 2) [18].

As only small amounts of HIBA are added in the electrolyte buffer (up to 0.01 M) and because of the moderate complex formation ability of HIBA with the cations, the terms of the second and third order in Eq. 14 with respect to the concentration of HIBA may be neglected. Further, we assume that $\mu_{\text{ML}} \beta_{\text{ML}} \alpha_L [\text{HL}] \ll \mu_0$, which means that μ_{ML} should be much smaller than μ_0 . Swaile and Sepaniak [14] calculated that the mobility of 1:1 Ca–HQS complexes was ten times smaller than that of the free Ca^{2+} .

In the absence of an organic solvent and a polyether, Eq. 14 can be rearranged into a linear form as

$$\frac{1}{\mu_{\text{eff}}} = \frac{1}{\mu_0} + \frac{\alpha_L \beta_{\text{ML}}}{\mu_0} \cdot [\text{HL}] \quad (16)$$

This is verified in Fig. 1b, where the reciprocal of μ_{eff} is plotted against the concentration of HIBA and a straight-line relationship is obtained for all of the divalent cations. μ_0 and β_{ML} can then be estimated from the straight lines.

4.2. Effect of methanol on migration

The experiments were performed at a HIBA concentration of 6.5 mmol/l and in the absence of 18-crown-6 (pH 4.5), while the fraction of methanol was changed from 0 to 40% (v/v). The maximum concentration of methanol used in this work was 40% (v/v) based on the following considerations: first, methanol is a volatile solvent, and evaporation during measurements can cause irreproducibility in analytical performance; and second, increasing the concentration of organic solvent decreases the electrical current and can cause electrical breakdown [25,26]. In this work, the electrical current was as low as 4.8 μA at 40% methanol.

Fig. 2a shows a plot of μ_{eff} as a function of

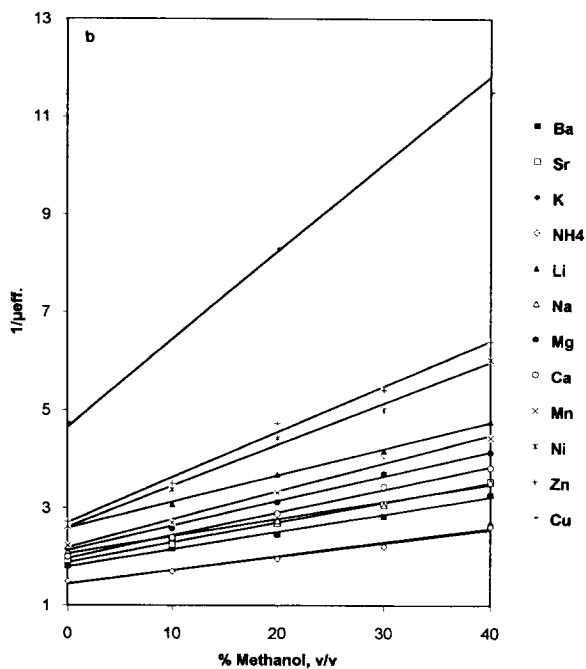
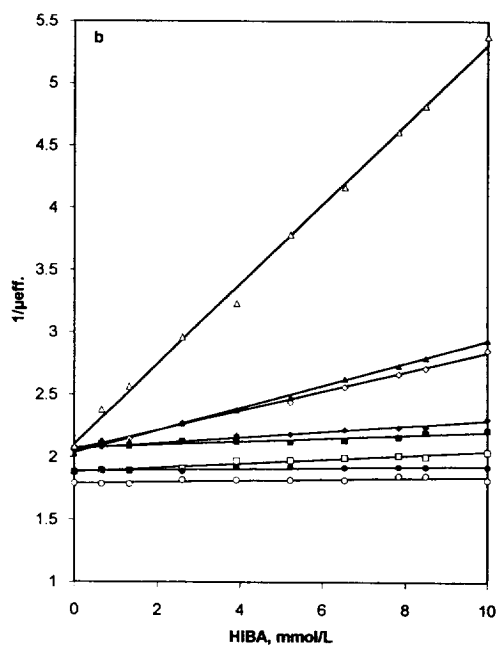
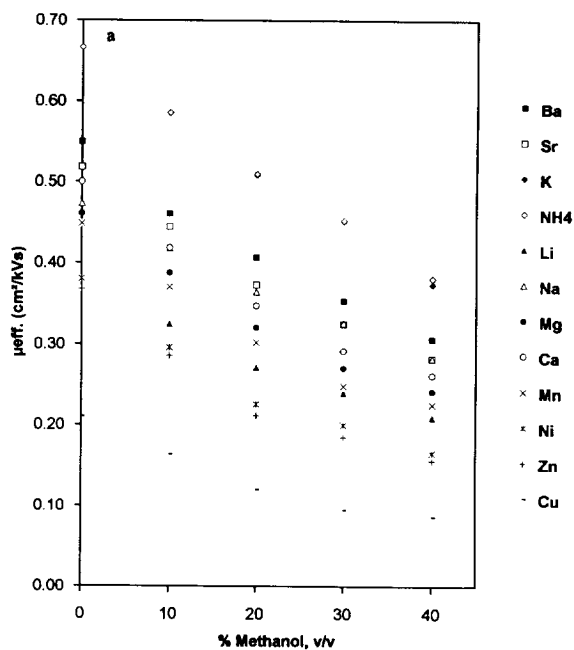
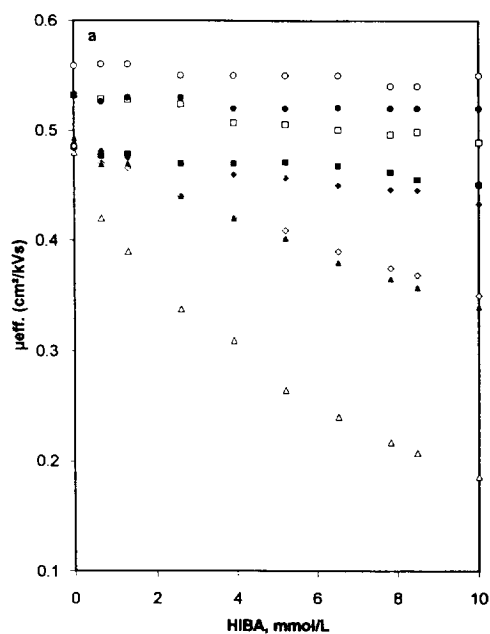


Fig. 1. (a) Electrophoretic mobility dependence of twelve inorganic cations on the concentration of HIBA. (b) Plot of reciprocal mobilities ($1/\mu_{\text{eff}}$) against concentration of HIBA. Experimental conditions: applied voltage, +20 kV; hydrostatic injection from a 10-cm height for 30 s; background electrolyte, 5 mmol/l imidazole (pH 4.5).

Fig. 2. (a) Electrophoretic mobility dependence of twelve inorganic cations on the fraction of methanol. (b) Plot of reciprocal mobilities ($1/\mu_{\text{eff}}$) against fraction of methanol. Experimental conditions: applied voltage +20 kV; hydrostatic injection from a 10-cm height for 30 s; background electrolyte; 5 mmol/l imidazole–6.5 mmol/l HIBA (pH 4.5).

methanol concentration. The electrophoretic mobilities of all the inorganic cations decrease as the fraction of methanol in water increases. For some of the divalent cations such as Cu^{2+} , Zn^{2+} , Ni^{2+} and Mn^{2+} , non-linear relationships are obvious. A similar effect of organic solvents on retention times has been observed in RPLC [27].

β_{MS} is expected to be small because the interaction between the cations and methanol is of the ion-dipole type [19], which is very weak (the calculated interaction constants are less than 0.1; see later). The mobilities of the solvated cations are expected to be smaller than those of the bare cations. Therefore, $\mu_{\text{MS}}\beta_{\text{MS}}[\text{S}]$ may be much smaller than μ_0 .

Accordingly, in the absence of a polyether, Eq. 14 can be rearranged in a linear form as

$$\frac{1}{\mu_{\text{eff}}} = \frac{1 + \alpha_{\text{L}} - \beta_{\text{ML}}[\text{HL}]}{\mu_0} + \frac{\beta_{\text{MS}}}{\mu_0} \cdot [\text{S}] \quad (17)$$

The term $1 + \alpha_{\text{L}} - \beta_{\text{ML}}[\text{HL}]$, is a constant as the pH and $[\text{HL}]$ were not changed in this set of experiments. This is verified in Fig. 2b, where a linear relationship between the reciprocal of μ_{eff} and methanol concentration is obtained for all of the cations. The values of β_{MS} can be estimated from the straight lines.

4.3. Effect of 18-crown-6 on migration

The experiments were conducted at a HIBA concentration of 6.5 mmol/l and at 20% (v/v) methanol (pH 4.5). As shown in Fig. 3a, the mobility of K^+ decreases continuously with an increase in the concentration of 18-crown-6, whereas the mobilities of Sr^{2+} and Ba^{2+} first decline rapidly and then level off when the concentration increases further, which must be due to saturation phenomena. The mobilities of the other cations are not significantly affected by the polyether. Na^+ is given as an example.

When plotting $1/\mu_{\text{eff}}$ of K^+ , Sr^{2+} and Ba^{2+} as a function of the 18-crown-6 concentration, curved lines are observed, suggesting that no simplification can be made (Fig. 3b).

From the above analysis, separately examining the migration dependence on HIBA, 18-crown-6

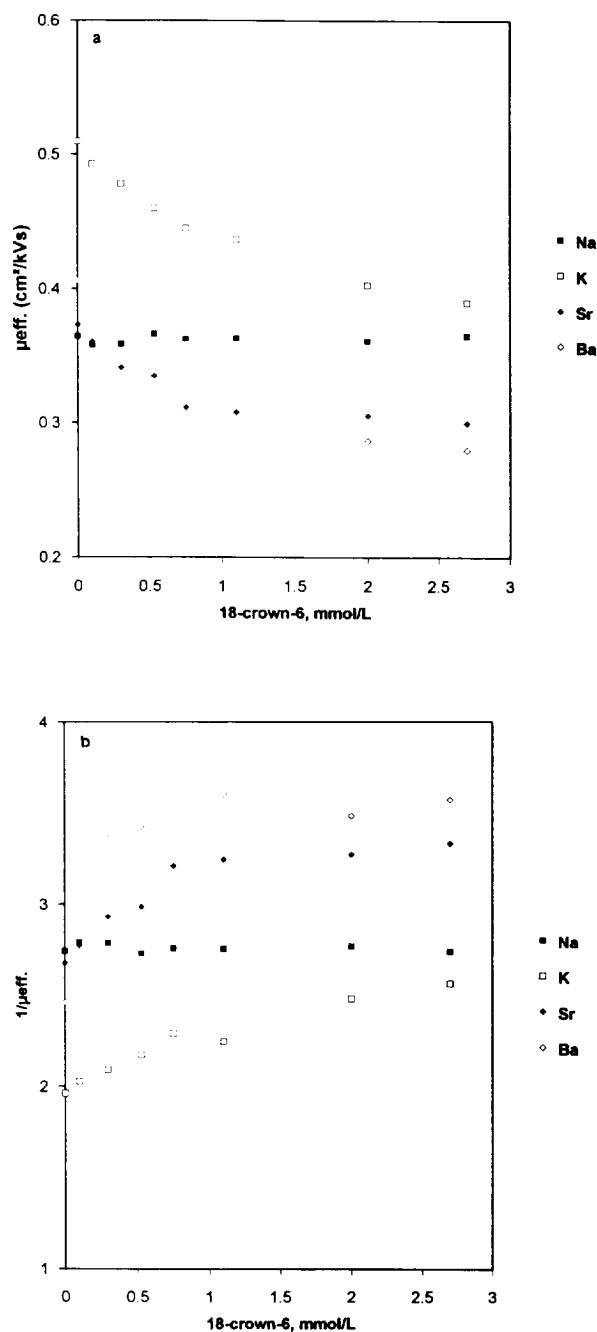


Fig. 3. (a) Electrophoretic mobility dependence of four inorganic cations on the concentration of 18-crown-6. (b) Plot of reciprocal mobilities ($1/\mu_{\text{eff}}$) against concentration of 18-crown-6. Experimental conditions: applied voltage +20 kV; hydrostatic injection from a 10-cm height for 30 s; background electrolyte, 5 mmol/l imidazole–6.5 mmol/l HIBA–20% (v/v) methanol (pH 4.5).

and methanol, the twelve inorganic cations are classified into three groups according to their electrophoretic behaviour and for each of the groups a characteristic migration model is derived from Eq. 14:

(1) Na^+ , NH_4^+ and Li^+ :

$$\mu_{\text{eff.}} = \frac{\mu_0}{1 + \beta_{\text{MS}}[\text{S}]} \quad (18)$$

Only methanol affects their electrophoretic mobilities.

(2) Mg^{2+} , Mn^{2+} , Ca^{2+} , Ni^{2+} , Zn^{2+} and Cu^{2+} :

$$\mu_{\text{eff.}} = \frac{\mu_0}{1 + \beta_{\text{ML}}\alpha_{\text{L}}[\text{HL}] + \beta_{\text{MS}}[\text{S}]} \quad (19)$$

Both HIBA and methanol influence their electrophoretic mobilities and manipulation of the concentration of the two reagents can subsequently modify the selectivity.

(3) Ba^{2+} , Sr^{2+} and K^+ :

$$\mu_{\text{eff.}} = \frac{\mu_0 + \beta_{\text{MC}}\mu_{\text{MC}}[\text{C}]}{1 + \beta_{\text{MC}}[\text{C}] + \beta_{\text{ML}}\alpha_{\text{L}}[\text{HL}] + \beta_{\text{MS}}[\text{S}]} \quad (20)$$

For K^+ , $\beta_{\text{ML}} = 0$. The mobilities of Ba^{2+} and Sr^{2+} are mainly affected by the polyether and by methanol. The effect of HIBA is only minor, as shown in Fig. 1.

4.4. Estimation of the parameters in the migration equations

The parameters in Eqs. 18–20 were estimated by non-linear regression. The initial values of the parameters required by the regression were obtained from the straight-line plots in Figs. 1b and 2b. We did not find a significant effect of the initial values on the estimation of the parameters.

The parameters are listed in Tables 1–4. Some literature values are also presented for comparison.

The calculated μ_0 is in good agreement with the experimentally obtained value. These values are lower than the values in the literature [28], but the sequence is the same as for those reported values. This may be due to the fact that

Table 1

Calculated electrophoretic mobilities (Calc.) compared with experimentally obtained mobilities (Exp.) and literature values [8,28]

Ion	$\mu_0(\text{cm}^2/\text{kV}\cdot\text{s})$			
	Calc. ^a	Exp.	Ref. [28]	Ref. [8]
Li^+	0.382 ± 0.012	0.382	0.402	0.39
Na^+	0.481 ± 0.011	0.473	0.521	0.48
NH_4^+	0.689 ± 0.019	0.667	0.764	
K^+	0.677 ± 0.021	0.688	0.764	0.68
Mg^{2+}	0.482 ± 0.005	0.485	0.551	
Ca^{2+}	0.532 ± 0.005	0.532	0.618	
Sr^{2+}	0.530 ± 0.006	0.532	0.616	
Ba^{2+}	0.559 ± 0.006	0.559	0.661	
Mn^{2+}	0.484 ± 0.006	0.488		
Ni^{2+}	0.485 ± 0.006	0.486		
Zn^{2+}	0.491 ± 0.008	0.493		
Cu^{2+}	0.470 ± 0.020	0.480		

^a In the calculation of the parameters, all the concentrations are in mol/l. Values given are means \pm S.E. ($n = 23$).

Table 2

Calculated formation constants ($\log \beta_{\text{ML}}$) of the inorganic cations with HIBA and literature values [18]

Ion	Calculated $\log \beta_{\text{ML}}^{\text{a}}$	Ref. [18]		
		$\log \beta_{\text{ML}}$	$\log \beta_{\text{ML}2}$	$\log \beta_{\text{ML}3}$
Mg^{2+}	0.89 ± 0.14	0.81	1.47	
Ca^{2+}	1.06 ± 0.09	0.92	1.42	
Sr^{2+}	0.46 ± 0.28	0.55	0.73	
Ba^{2+}	0.59 ± 0.21	0.36	0.51	
Mn^{2+}	1.17 ± 0.07	0.96	1.54	1.56
Ni^{2+}	1.69 ± 0.04	1.67	2.80	2.84
Zn^{2+}	1.76 ± 0.04	1.71	3.01	
Cu^{2+}	2.31 ± 0.06	2.74	4.34	4.38

^a See Table 1.

Table 3

Calculated formation constants ($\log \beta_{\text{MC}}$) of the inorganic cations with 18-crown-6 and literature values [31,32] and the mobilities of these ions bound to 18-crown-6 (μ_{MC})

Ion	$\log \beta_{\text{MC}}^{\text{a}}$	Ref. [31]	Ref. [32]	μ_{MC}
K^+	2.86 ± 0.30	2.03	2.1	0.31
Sr^{2+}	3.35 ± 0.20	2.72	2.9	0.28
Ba^{2+}	4.27 ± 0.13	3.87	4.1	0.28

^a See Table 1.

Table 4
Calculated interaction constants (β_{MS}) of the inorganic cations with methanol and ionic radii [28]

Ion	β_{MS}^a	$r(\text{pm})$ [28]
NH_4^+	0.077 ± 0.007	
Li^+	0.079 ± 0.008	60
Na^+	0.067 ± 0.006	95
K^+	0.071 ± 0.009	133
Mg^{2+}	0.095 ± 0.005	65
Ca^{2+}	0.098 ± 0.004	99
Sr^{2+}	0.082 ± 0.004	113
Ba^{2+}	0.076 ± 0.004	135
Mn^{2+}	0.109 ± 0.006	91
Ni^{2+}	0.168 ± 0.009	70
Zn^{2+}	0.189 ± 0.015	74
Cu^{2+}	0.366 ± 0.091	72

^a See Table 1.

μ_0 in our case was obtained in an electrolyte buffer with an organic compound (5 mmol/l imidazole). This buffer may have a higher viscosity than pure water. As the electrophoretic mobility is inversely proportional to the viscosity of the medium, a decreased mobility can result. A lower mobility was also observed by Vogt and Conradi [16] and ascribed to the adsorption of non-complexed cations on the negatively charged capillary wall. In fact, the electrophoretic mobility usually found in standard tables [28,29] is a physical constant determined for full solute charge and extrapolated to infinite dilution. The calculated mobilities for Li^+ , Na^+ and K^+ are in good agreement with the values reported by Beck and Engelhardt [8]. They obtained the mobilities in a 5 mmol/l imidazole background electrolyte buffer at pH 4.5 in CIE.

The calculated formation constants of the cations with HIBA (from Eqs. 18–20) are given in Table 2, and are in good agreement with the literature values [18]. The larger deviation in the formation constants of Ba^{2+} and Sr^{2+} with HIBA may be due to the fact that their effective mobilities change very slightly on adding HIBA to the electrolyte buffer.

All the experiments were performed at pH 4.5, where sufficient protonation of imidazole and dissociation of HIBA are guaranteed. In addition, the formation of hydroxides of the

metal cations studied is negligible at this pH. Moreover, adsorption on the capillary wall may not be significant. The mole fraction of the conjugated base of HIBA, α_{L^-} , is calculated according to Eq. 6 using a $\text{p}K_a$ of 3.971 [10] and equals 0.772 at pH 4.5. One should be aware that the real $\text{p}K_a$ value is expected to be higher in a binary medium than in pure water [30], so the real mole fraction of L^- may be lower than 0.772. However, it was not found to cause a significant deviation, probably because the effect of pH on the cation mobility is secondary.

The calculated formation constants of K^+ , Sr^+ and Ba^{2+} with 18-crown-6 ether are given in Table 3. The calculated values are higher than those reported in the literature [31,32]. On comparing Tables 1 and 3, it is seen that the cations bound to 18-crown-6 move towards the cathode at a mobility lower than the bare ones; this accounts for the decrease in effective mobility. It is also seen in Table 3 that the bound Sr^{2+} and Ba^{2+} have the same mobilities.

In Table 4, the calculated interaction constants between methanol and the cations along with the ionic radius obtained from Ref. [28] are listed. It is seen that within the same column of the Periodic Table (IA and IIA), the constants tend to decrease with increasing ionic radius. For group B, the constants tend to increase with decreasing radius. To our knowledge, these constants have not been reported previously. Fig. 4 shows a good correlation for the divalent cations between their formation constants with HIBA and their interaction constants with methanol.

4.5. Description of migration behaviour and optimization of separation

The validity of the proposed model was confirmed by comparing the calculated electrophoretic mobilities of the twelve cations with the observed values shown in Fig. 5. The slope of the best fit of the data computed with linear regression is 0.9978 ± 0.0081 , which is not significantly different from 1; the intercept is 0.0009 ± 0.0034 , which is not significantly different from 0. The line in Fig. 5 represents the case

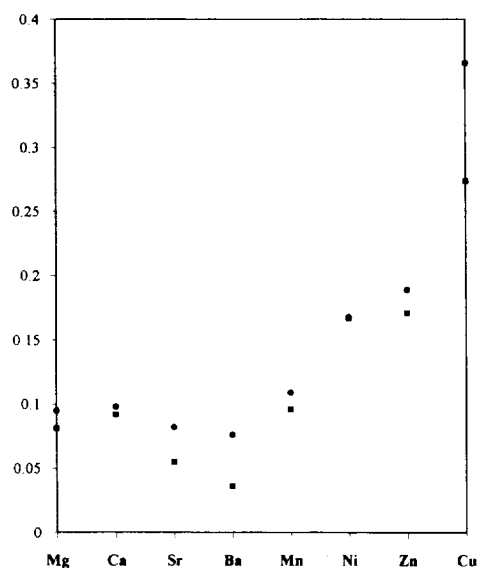


Fig. 4. Comparison for the divalent inorganic cations of the interaction constants with methanol and the formation constants with HIBA. ● = β_{MIS} ; ■ = $0.1 \cdot \log \beta_{ML}$.

of perfect correlation. One concludes that the migration model represents the experimental data well.

Having obtained a migration model, one can

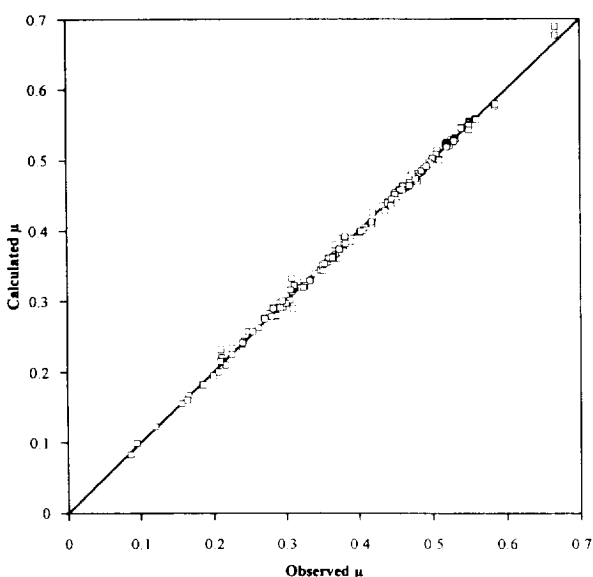


Fig. 5. Correlation plot of observed and predicted ionic mobilities of the cations (μ in $\text{cm}^2/\text{kV}\cdot\text{s}$).

try to predict the optimum separation in the whole experimental domain. To find the optimum experimental conditions, an optimization criterion is necessary. Different criteria have been used to find the optimum in capillary electrophoresis, such as the resolution between two adjacent peaks, R_s , the mobility ratio between two neighbouring solutes, α , and the mobility difference between two adjacent peaks, $\Delta\mu$, which is proportional to resolution. As peak cross-over is expected in this study, the absolute mobility difference is used. The optimization criterion is a mini-max criterion: one determines the absolute mobility difference of the worst separated pairs of ions, $|\Delta\mu_{\min}|$, at each possible combination of the three variables and then determines the combination of the three variables for which a maximum $|\Delta\mu_{\min}|$ is found.

Fig. 6a shows a contour plot of $|\Delta\mu_{\min}|$ as a function of the concentration of HIBA and methanol concentration. It is seen that the higher $|\Delta\mu_{\min}|$ values are located at higher concentrations of HIBA and medium methanol concentration. Fig. 6b shows a contour plot of $|\Delta\mu_{\min}|$ as a function of the concentration of 18-crown-6 and methanol concentration at 6.5 mmol/l HIBA. In regions A and B, the best separations are predicted. Fig. 7 shows the separation of the twelve inorganic cations under the experimental conditions close to A, namely 6.5 mmol/l HIBA, 0.53 mmol/l 18-crown-6 and 20% (v/v) methanol. Baseline separation of the cations, including one additional ion (Cr^{3+}), is achieved.

As seen from Fig. 6b, when the methanol concentration is kept constant, $|\Delta\mu_{\min}|$ does not change significantly if the concentration of 18-crown-6 increases over ca. 1 mmol/l. This is due to the mobilities of Sr^{2+} and Ba^{2+} having reached a more or less constant value in that the chemical equilibrium is saturated. Further increases in the concentration of 18-crown-6 only change the K^+ mobility, which does not influence the separation. On the other hand, on increasing the fraction of methanol while keeping the concentration of 18-crown-6 constant, $|\Delta\mu_{\min}|$ changes in a non-continuous fashion. This is because the mobilities of Na^+ , Ca^{2+} ,

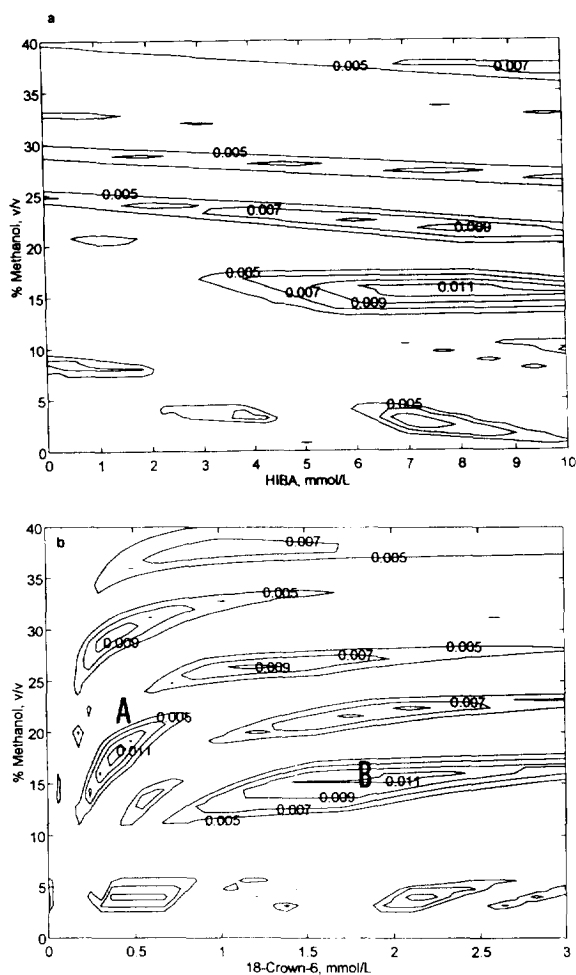


Fig. 6. (a) Plots presenting $|\Delta\mu_{\min}|$ as a function of (a) methanol concentration and HIBA concentration and (b) methanol concentration and 18-crown-6 concentration. The lines delimit areas of equal $|\Delta\mu_{\min}|$.

Mg^{2+} , Mn^{2+} , Li^+ , Ni^{2+} and Zn^{2+} decrease with increasing fraction of methanol, so that these cations approach and cross over Sr^{2+} and Ba^{2+} , for which the mobilities are unchanged (see Fig. 8). Therefore, the concentration of methanol is critical for the separation and one should control this variable accurately to obtain a rugged separation.

Using the proposed model, the effect of methanol on the complexation of the inorganic cations with HIBA and 18-crown-6 can be simulated. As seen from Fig. 6, at high methanol concentrations (from 35 to 40%), $|\Delta\mu_{\min}|$ hardly

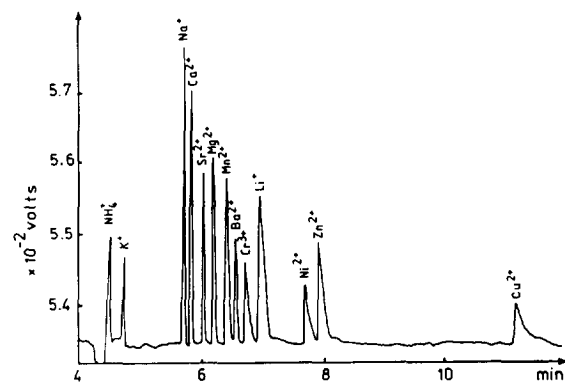


Fig. 7. Separation of a mixture containing NH_4^+ , K^+ , Na^+ , Li^+ , Mg^{2+} , Ca^{2+} and Cr^{3+} ($2 \mu\text{g/ml}$), Sr^{2+} , Ba^{2+} , Mn^{2+} , Ni^{2+} and Zn^{2+} ($4 \mu\text{g/ml}$) and Cu^{2+} ($6 \mu\text{g/ml}$). Experimental conditions: applied voltage +20 kV; hydrostatic injection from a 10-cm height for 20 s; $I \approx 6.0 \mu\text{A}$; $T \approx 23^\circ\text{C}$; background electrolyte 5 mmol/l imidazole–6.5 mmol/l HIBA–20% (v/v)–methanol and 0.53 mmol/l 18-crown-6 (pH 4.5).

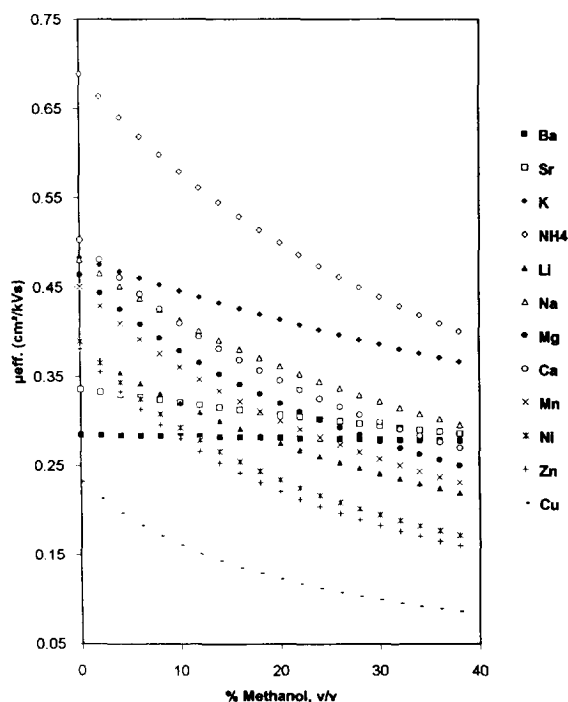


Fig. 8. Simulated change in electrophoretic mobilities of twelve inorganic cations as a function of methanol concentration at 6.5 mmol/l HIBA and 1.5 mmol/l 18-crown-6.

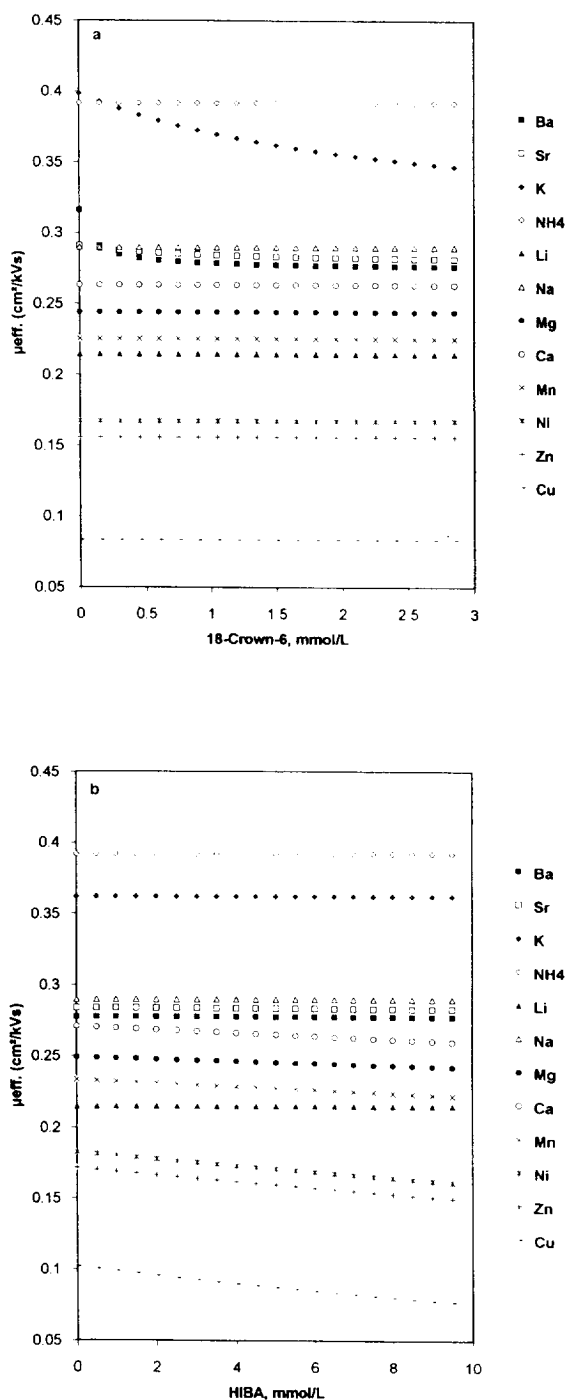


Fig. 9. Simulation of migration behaviour of twelve inorganic cations at a high fraction of methanol in water (40%, v/v) as a function of (a) 18-crown-6 concentration (6.5 mmol/l HIBA) and (b) HIBA concentration (1.5 mmol/l 18-crown-6).

changes. This is seen more clearly in Fig. 9a. At 40% methanol, the mobilities of Sr^{2+} and Ba^{2+} change slightly at concentrations of 18-crown-6 less than ca. 0.3 mmol/l, but they remain constant as the concentration of 18-crown-6 increases further. Sr^{2+} and Ba^{2+} are located between Na^+ and Ca^{2+} . The Ba–Sr or Na–Sr distance determines $|\Delta\mu_{\text{min}}|$, which, however, does not vary any more in the presence of high concentrations of methanol. The selectivity gained from the inclusion complexation is therefore lost in this situation.

A similar effect was observed for HIBA, as shown in Fig. 9b, where the mobilities of the divalent cations do not change or change only slightly with increasing concentration of HIBA. Probably, methanol molecules interact with the cations in a direct way and most of the cations may have been solvated by the solvent molecules. The solvation competes for the cations with HIBA and 18-crown-6, resulting in a decrease in the apparent formation constants of the cations with HIBA and 18-crown-6. In this case, the selectivity is determined by methanol, which is insufficient, however, to separate all of the cations. We did observe this in a separate experiment, where there was no separation of Sr^{2+} and Na^+ in the presence of 6.5 mmol/l HIBA and 40% methanol. Therefore, one should avoid too high a fraction of methanol when relatively weak complexing reagents are used.

5. Conclusions

Based on multiple chemical equilibria, a migration model was designed that describes the migration of inorganic cations in terms of absolute mobility, complex formation constants, the concentrations of complexing reagents and the fraction of organic solvent in the electrolyte buffer. This electrolyte buffer was composed of HIBA, 18-crown-6 and methanol together with a UV-absorbing substance, imidazole. Good agreement was achieved between theoretical predictions and experimental data. By applying this model, the separation of twelve inorganic cations was optimized. The selectivity of the separation

gained from complexation deteriorated when the ratio of methanol to water in the electrolyte buffer was too high (40%, v/v), as methanol decreases the apparent complex formation constants.

References

- [1] P. Jandik, W.R. Jones, A. Weston and R.P. Brown, *LC·GC*, 5 (1991) 20.
- [2] P.E. Jackson and P.R. Haddad, *Trends Anal. Chem.*, 12 (1993) 231.
- [3] P. Jandik and G. Bonn, *Capillary Electrophoresis of Small Molecules and Ions*, VCH, New York, 1993.
- [4] C.A. Monning and R.T. Kennedy, *Anal. Chem.*, 66 (1994) 280R.
- [5] W.G. Kuhr and E.S. Yeung, *Anal. Chem.*, 63, (1991) 275A.
- [6] F. Foret, S. Fanali, L. Ossicini and P. Bocek, *J. Chromatogr.*, 470 (1989) 299.
- [7] V. Sustacek, F. Foret and P. Bocek, *J. Chromatogr.*, 545 (1991) 239.
- [8] W. Beck and H. Engelhardt, *Chromatographia*, 33 (1992) 313.
- [9] Q. Yang, M. Jimidar, T. Hamoir, J. Smeyers-Verbeke and D.L. Massart, *J. Chromatogr. A*, 673 (1994) 275.
- [10] Q. Yang, J. Smeyers-Verbeke, W. Wu, M.S. Koths and D.L. Massart, *J. Chromatogr. A*, 688 (1994) 339.
- [11] A. Weston, P.R. Brown, A.L. Heckenberg, P. Jandik and R. Jones, *J. Chromatogr.*, 602 (1992) 249.
- [12] S. Motomizu, S. Nishimura, Y. Obata and H. Tanaka, *Anal. Sci.*, 7 (1991) 253.
- [13] T. Hirokawa, N. Aoki and Y. Kiso, *J. Chromatogr.*, 312 (1984) 11.
- [14] D.F. Swaile and M.J. Sepaniak, *Anal. Chem.*, 63 (1991) 179.
- [15] C. Quang and M.G. Khaledi, *J. Chromatogr. A*, 659 (1994) 459.
- [16] C. Vogt and S. Conradi, *Anal. Chim. Acta*, 294 (1994) 145.
- [17] R.S. Sahota and M.G. Khaledi, *Anal. Chem.*, 66 (1994) 1141.
- [18] J. Inczédy, in J. Tyson (Editor), *Analytical Application of Complex Equilibria*, Ellis Horwood, Chichester, 1976, p. 347.
- [19] K. Bächmann, J. Boden and I. Haumann, *J. Chromatogr.*, 626 (1992) 259.
- [20] K. Fukushi and K. Hiuro, *J. Chromatogr.*, 523 (1990) 281.
- [21] R.T. Morrison and R.N. Boyd, *Organic Chemistry*, Allyn and Bacon, Boston, MA, 5th ed., 1987, ch. 6, p. 223.
- [22] T. Okada and T. Usui, *Anal. Chem.*, 66 (1994) 1654.
- [23] *SPSS/PC + Statistics 4.0*, SPSS, IL, 1990.
- [24] *Matlab for Microsoft Windows*, MathWorks, 1992.
- [25] I.M. Johansson, E.C. Huang, J.D. Henion and Z. Weigenbaum, *J. Chromatogr.*, 554 (1991) 331.
- [26] G.M. Janini, K.C. Chan, J.A. Barnes, G.M. Muschik and H.J. Issak, *Chromatographia*, 35 (1993) 497.
- [27] P.J. Schoenmakers and R. Tijssen, *J. Chromatogr. A*, 656 (1993) 577.
- [28] R.C. Weast (Editor), *CRC Handbook of Chemistry and Physics*, CRC Press, Boca Raton, FL, 67th ed., 1986.
- [29] D.N. Heiger, *High Performance Capillary Electrophoresis—An Introduction*, Hewlett-Packard, Waldbronn, 1992, ch. 2, p. 15.
- [30] H. Freiser, *Concepts and Calculations in Analytical Chemistry. A Spreadsheet Approach*, CRC Press, Boca Raton, FL, 1992, ch. 2, p. 34.
- [31] R.M. Smith and A.E. Martell, *Critical Stability Constants*, Plenum Press, New York, 1975.
- [32] R.M. Izatt, K. Pawlak, J.S. Bradshaw and R.L. Bruening, *Chem. Rev.*, 91 (1991) 1721.