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Simultaneous separation of ammonium and alkali, alkaline earth and transition metal ions in aqueous–organic media by capillary ion analysis

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Abstract

The simultaneous separation of the mono-, di- and trivalent cations NH_4^+, K^+, Na^+, Li^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+} and Cr^{3+} by capillary ion analysis was studied in a background electrolyte system composed of imidazole, 2-hydroxyisobutyric acid, 18-crown-6 and methanol. The effects of this system on the electroosmotic and electrophoretic mobility were studied in detail. A competitive complexation between 2-hydroxyisobutyric acid and sulphuric acid was observed which determined the cation mobility. Applying a central composite design, a quantitative description of the electrophoretic behaviour of the inorganic cations as a function of the system parameters was made and the optimum separation could be predicted. It was found in most instances that a linear model is sufficient for mobility modelling. Using a full factorial design, the main effects on the electroosmotic flow were estimated and methanol was found to be the dominating factor. The applicability of the capillary electrophoresis method was examined by separating ions in Chinese tea infusions. Five metal cations (K^+, Na^+, Ca^{2+}, Mg^{2+} and Mn^{2+}) were detected. A limit of detection at the μg/l level could be achieved using electromigration injection.

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1. Introduction

Different types of capillary electrophoresis (CE), namely capillary zone electrophoresis (CZE) [1,2], micellar electrokinetic capillary chromatography (MEKC) [3,4] and isotachophoresis (ITP) [5], have been applied to the separation of inorganic cations. Among these, CZE is the simplest. By adding a complexing reagent to the electrolyte buffer, such as 2-hydroxyisobutyric acid (HIBA) [6,7], 8-hydroxyquinoline-5-sulfonic acid [2] or 1,2-cyclohexanediamine-N,N,N,N′-tetraacetic acid (CyDTA) [8], efficient separation of metal cations has been achieved. The separation is based on the difference in complex formation between individual cations and the ligand. The larger the formation constant, the lower is the apparent charge of a complexed cation and therefore the slower its movement.

Foret et al. [6] proposed a CZE method for the separation of lanthanides which employed a mixture of creatinine and HIBA as a background electrolyte (BGE). Later, Weston et al. [7]

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developed the method for separating alkali, alkaline earth and transition metal cations. Beck and Engelthardt [9] proposed imidazole–H_2SO_4 as the BGE system for the separation of alkali and alkaline metal cations. The method was successfully adapted to the quantitative analysis of pharmaceutical electrolyte solutions and fruit juices, and compared with atomic spectrometry in our previous work [10]. Recently, Shi and Fritz [11] investigated the use of lactate, phthalate and tartrate as complexing reagents for CZE separation of metal cations.

A special complexation mechanism is the formation of inclusion complexes by using crown ethers, such as 18-crown-6 and 15-crown-5. They have been used in the CE separation of alkali and alkaline earth metal cations and some transition metal cations [5,12,13]. Use of 18-crown-6 has also been considered in the chiral separation of organic compounds [14]. The crown ether can strongly bind K^+ through its specific cyclic ring structure, lined inside with oxygen atoms carrying unshared pairs of electrons. Therefore, it is added to the BGE to moderate the mobility of K^+. As a result, K^+ and NH_4^+ are separated.

The addition of organic solvents for the CE analysis of organic compounds has been proposed by several groups [15,16]. The organic solvents are added to the running buffer to improve analyte solubility and detection sensitivity, to control electroosmotic flow (EOF) and to provide an additional selectivity. Motomizu et al. [8] have shown the effects of organic solvents such as acetone, ethanol, ethylene glycol and hexanesulfonic acid on the CZE separation of metal–CyDTA complexes. Recently, Janini et al. [15] confirmed that the addition of organic solvents to the electrolyte buffer causes a large change in buffer viscosity. This change is responsible for variations in electroosmotic mobility (μ_{eof}) and electrophoretic mobility (μ_{ep}). The influence on μ_{ep} depends on the extent of solute dissociation and therefore is pH dependent in an acid–base equilibrium system. So far, the CZE separation of metal cations has rarely been considered in mixed organic aqueous media. We decided to study this possibility in more detail.

In this work, we studied an aqueous–organic BGE system composed of imidazole, HIBA, 18-crown-6 and methanol, investigating the effects of the important system parameters on migration and EOF. In addition to a one-variable-at-a-time optimization approach, modelling the electrophoretic behaviour of the inorganic cations as a function of the concentrations of HIBA, 18-crown-6 and methanol was investigated. A central composite design was applied to determine the model coefficients accurately and to predict the optimum separation. The practical applicability of the CE method was evaluated by separating ions in Chinese tea infusions.

2. Experimental

2.1. 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 either by hydrostatic or electromigration injection. The electropherograms were recorded and treated with a Waters 810 data workstation equipped with a W51 watch-dog interface. Temperature control was carried out as described in Ref. [10]. The SPSS program [17] was used with an IBM PC2 computer for statistical calculations.

2.2. Capillary preparation and cleaning

Every morning, the capillary was washed for 1 min with 0.5 *M* KOH, for 2 min with water obtained from a Milli-Q system (Millipore) (Milli-Q water) 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 each with 0.1 *M* KOH and Milli-Q water and for 3 min with the electrolyte buffer. At the end of the day, the capillary was rinsed with Milli-Q water for 5 min and left in the water.

2.3. *Reagents and standards*

Water used for the preparation of all solutions was obtained from a Milli-Q water purification system and contained no detectable analyte cations.

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 or nitrate 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 was of analytical-reagent grade and methanol of chromatography grade. H_2SO_4 and HCl were of Suprapur grade (Merck), 2-Hydroxyisobutyric acid and 18-crown-6 were 99% pure reagents (Aldrich).

2.4. *Preparation of background electrolyte*

First, three stock standard solutions containing 130.6 m*M* HIBA, 50.0 m*M* 18-crown-6 and 500.0 m*M* imidazole were prepared. The background electrolytes used in CZE analysis were prepared by mixing appropriate amounts of these stock standard solutions and corresponding volumes of reagent methanol in a 100-ml plastic volumetric flask. The pH was then adjusted by titration first with 5 *M* and subsequently with 0.5 *M* H_2SO_4. For all the BGEs, the pH was kept at ca. 4.5. The electrolyte solutions were stored in a refrigerator. Just before use they were filtered through a 0.45-μm syringe filter (Millipore, Molsheim, France). In modelling mobility experiments, the dilute H_2SO_4 was replaced with 1 *M* HCl for the adjustment of the buffer pH.

2.5. *Samples*

Chinese green and black teas were bought in a local tea shop in China. The tea infusions were prepared in the following way: ca. 0.5 g of each of teas was weighed in quartz cups and infused in 15 ml of boiling Milli-Q water for 10 min. The infusions were then filtered through a 0.45-μm syringe filter. For the analysis, the solutions were diluted two-fold (1 + 1). The green tea infusion was spiked as follows: 1 ml of the tea infusion and 6 ml of 100 μg/ml ammonium nitrate were pipetted in a 100-ml flask and diluted to volume with Milli-Q water. The solution was again diluted sixfold with Milli-Q water.

3. Results and discussion

3.1. *Competitive complexation effect between HIBA and sulfate*

Fig. 1 is a plot of cation mobilities as a function of the concentration of HIBA. For

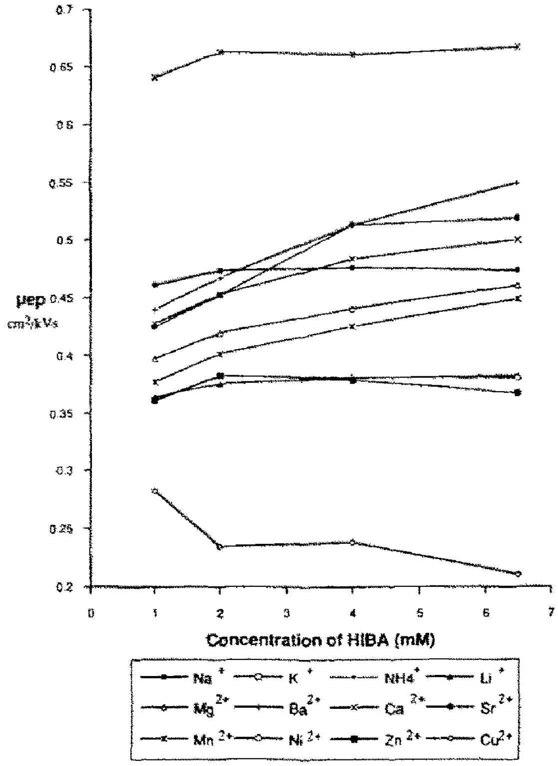

Fig. 1. Dependence of the ionic mobility μ_{ep} on the concentration of HIBA. 2 μg/ml each of NH_4^+, K^+, Na^+, Li^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+} and Cu^{2+}. Experimental conditions: applied voltage, +20 kV; hydrostatic injection from 10 cm height for 30 s; BGE, 5 m*M* imidazole at pH 4.5.

NH_4^+, Li^+ and K^+, the mobilities are hardly affected by HIBA because they do not form any complexes with HIBA [7] and also have very small formation constants with sulfate [16]. For the alkaline earth metal cations Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} the mobilities increase as the HIBA concentration increases. This is also observed for the transition metal cation Mn^{2+}. This observation, however, is contradictory to that in the UVCat1-HIBA BGE, where the mobility decreased with increasing HIBA concentration [7]. This is a result of the competition between sulfate and HIBA for the cations. The effective electrophoretic mobility of each resulting CZE band is regulated by the relative contribution of the various possible chemical forms, such as free metal cation and metal–HIBA and metal–sulfate complexes. As the concentration of HIBA increases there is more $M(HIBA)^+$ complex and less uncharged sulfate complex, so the mobility increases. For Cu^{2+}, Zn^{2+} and Ni^{2+}, which have high formation constants (Table 1 [18]), probably $M(HIBA)_2^0$ is formed. Therefore, a decrease in the mobilities of Zn^{2+}, Ni^{2+} and Cu^{2+} is noticed in Fig. 1. As the HIBA complexation becomes more important with increasing concentration, the selectivity of the separation increases as the formation constants of HIBA complexes are more different for different ions than the sulfate complexes. As we shall see later, by replacing H_2SO_4 with HCl for BGE pH adjustment, a linear mobility dependence on the HIBA concentration is experimentally confirmed. Bächmann et al. [12] also observed the effect of SO_4^{2-} on the ionic mobility in a $Ce_2(SO4)_3$–18-crown-6 BGE system (indirect fluorimetric detection) and found differences of up to 20% between the measured mobilities and the values cited in the literature [19], attributing this to the formation of sulfate salts.

Table 1
Logarithm of overall complex formation constants (β) of HIBA and sulfate with cations [18]

Cation	Log β	
	HIBA	SO_4^{2-}
Mg^{2+}	MgL 0.81, MgL_2 1.47	2.25
Ca^{2+}	CaL 0.92, CaL_2 1.42	2.30
Sr^{2+}	SrL 0.55, SrL_2 0.73	2.31
Ba^{2+}	BaL 0.36, BaL_2 0.51	2.55
Mn^{2+}	MnL 0.96, MnL_2 1.54	2.26
Zn^{2+}	ZnL 1.71, ZnL_2 3.01	2.38
Ni^{2+}	NiL 1.67, NiL_2 2.85, NiL_3 2.84	2.32
Cu^{2+}	CuL 2.74, CuL_2 4.34, CuL_3 4.05	2.36

Fig. 2 shows a plot of μ_{eof} as a function of the concentration of HIBA. μ_{eof} decreases when the concentration of HIBA in the BGE increases (without the addition of 18-crown-6 and methanol). With 1 m*M* HIBA, $\mu_{eof} = 0.48$ $cm^2/kV \cdot s$ and the relative standard deviation (R.S.D.) of the μ_{eof} calculated from 15 independent measurements is 4%. The μ_{eof} and R.S.D. decline rapidly to 0.42 $cm^2/kV \cdot s$ and 2% ($n = 15$), respectively, when the concentration of HIBA is increased to 2 m*M*. A further increase in the HIBA concentration to 6.5 m*M* does not change μ_{eof} significantly but results in an R.S.D. of 1%. We conclude that sufficient amounts of HIBA can improve the stability of the capillary surface.

3.2. *Effect of methanol*

HIBA provides an efficient selectivity of the separation for most of the cations studied here, but as shown in Fig. 3a, the separation between Li^+, Ni^{2+} and Zn^{2+} is insufficient even at 6.5 m*M* HIBA. A further increase in HIBA concentration was found to produce an interference

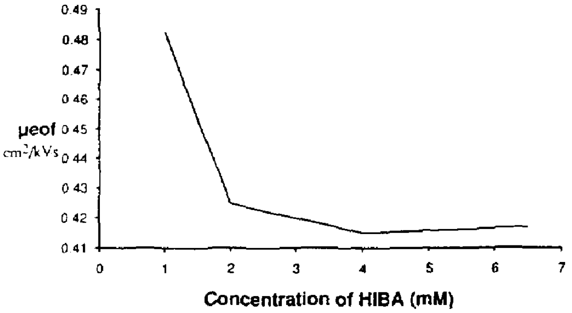

Fig. 2. Dependence of electroosmotic mobility μ_{eof} on the HIBA concentration. Experimental conditions as in Fig. 1.

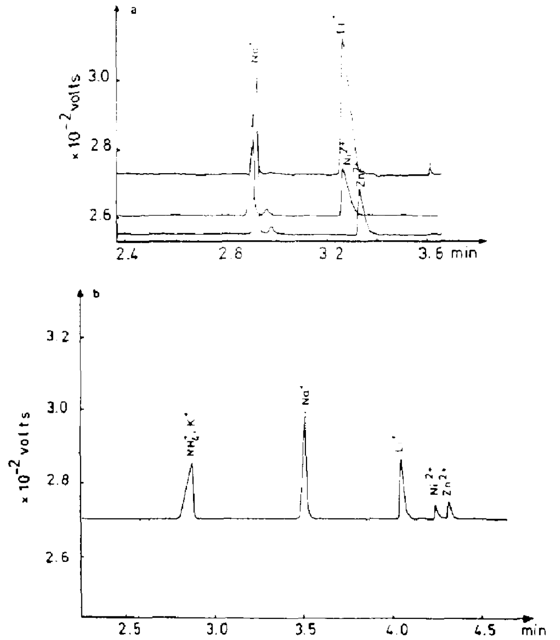

Fig. 3. Separation of Li^+, Ni^{2+} and Zn^{2+} (a) without methanol and (b) with 10% (v/v) methanol in the BGE. Experimental conditions: applied voltage, +20 kV; hydrostatic injection from 10 cm height for 30 s; BGE, 5 m*M* imidazole–6.5 m*M* HIBA at pH 4.5.

peak, which can interfere with the other cations. The cause is not clear. For this reason, the amount of HIBA in the BGE was not increased further. As shown in Fig. 3b, however, by adding 10% (v/v) methanol to the electrolyte buffer, the mobilities of Ni^{2+} and Zn^{2+} are greatly decreased, so that they are separated from Li^+.

The effect of methanol on the electrophoretic mobility of the cations was investigated systematically. This was carried out by changing the percentage of methanol in the BGE up to 30% (v/v) while keeping the concentration of HIBA constant. The resulting plot is given in Fig. 4. With increasing percentage of methanol, the mobilities of all the cations decrease almost linearly and elution of the cations is greatly delayed. Methanol affects Li^+ and Na^+ comparatively less, so that peak cross-over occurs when the amount of methanol in the BGE is continuously increased. It follows that methanol modifies the selectivity of CZE separation for inorganic cations.

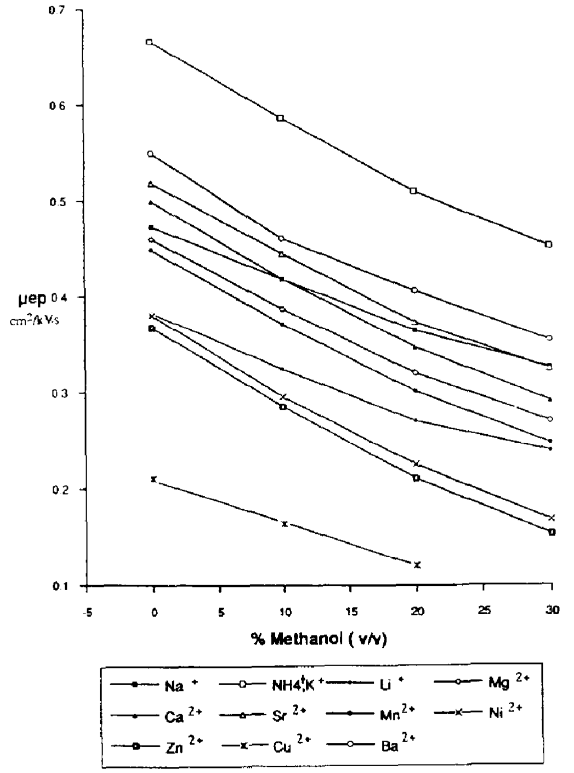

Fig. 4. Dependence of the ionic mobility μ_{ep} on the methanol concentration in the BGE. 2 μg/ml each of NH_4^+, K^+, Na^+, Li^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+} and Cu^{2+}. Experimental conditions as in Fig. 3.

However, overuse of methanol can produce a negative effect on the separation efficiency owing to broadening of the peak shape. Others [20] also argued that large amounts of organic solvents can cause electrical breakdown and have no advantages except for the improvement of the solubility of hydrophobic compounds. Therefore, 30% (v/v) methanol in the BGE is the maximum amount we studied.

As expected, a strong effect of methanol on μ_{eof} was also observed. Fig. 5 shows that an increase in the methanol content results in a decrease in μ_{eof} when the concentration of HIBA in the BGE is kept constant. The stability of μ_{eof} does not change significantly since the R.S.D. values are always below 1%. The excel-

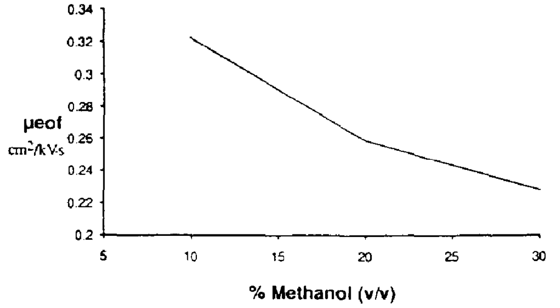

Fig. 5. Dependence of electroosmotic mobility μ_{eof} on the methanol concentration. Experimental conditions as in Fig. 3.

lent repeatability of μ_{eof} is indicative of the stability of the capillary surface. This is one of the important factors in ensuring a repeatable electrophoretic performance. At 30% methanol, $\mu_{eof} = 0.23$ cm^2/kV·s, which is about half of that in the absence of methanol. Comparing Figs. 4 and 5, the electroosmotic mobility (μ_{eof}) is smaller than the ionic mobility in most instances. The decline in the EOF is attributed mainly to the change in buffer viscosity caused by methanol [15].

Organic solvents may influence electrophoretic behaviour: (i) change the viscosity of the electrolyte buffer; (ii) solvate the solutes [15]; and (iii) affect the chemical equilibrium [21,22].

(i) A change in the viscosity of the electrolyte buffer causes a variation in μ_{ep}. The effects of organic solvents can be derived from the following equation [23]:

$$\mu_{ep} = q/6\pi\eta r \tag{1}$$

where q is the net charge of the solute, r the Stokes radius and η the viscosity of the medium. According to Janini et al. [15], an increase in the viscosity of the medium, η, resulting from the addition of organic solvents, leads to a decrease in μ_{ep}. The effect on μ_{ep} also depends on the charge and size of the solute.

(ii) The influence of organic solvents on the migration can also occur through the ion–dipole bonds that exist between a charged ion and a polar solvent molecule. The solvated cations are altered in geometry and therefore their movement in the electrical field is slowed. According to Freiser [21], depending on the specific properties of the ions and the solvent molecules, the size and type of solvation shell around the ions will differ and therefore the mobilities of the ions are altered differently. The ultimate result is a maximization of the separation due to the increased mobility difference.

(iii) There is an alternative view on the effect of the solvents. Methanol has a lower dielectric constant than water. The lower the dielectric constant of a medium, the easier it is for ions to be associated in the medium. This means that a complexation reaction, where charge neutralization occurs, will be favoured by a decrease in the dielectric constant, and the formation of high-degree complexes, e.g., $M(HIBA)_2^0$, can be promoted. This then decreases the cation mobility and provides an additional possibility for improving the separation.

3.3. *Effect of 18-crown-6*

K^+ and NH_4^+ have almost the same mobility, which is not significantly affected by HIBA and methanol. Two approaches have been used to facilitate their separation, increasing the pH of the BGE to decrease the mobility of NH_4^+ [24] or employing inclusion complexation by adding 18-crown-6 [12,13]. Fig. 6 shows the variation of the cation mobility as the concentration of 18-crown-6 changes from 0 to 2.7 m*M*, at 6.5 m*M* HIBA, pH 4.5 and 20% (v/v) methanol. A sharp decline in the mobility of K^+, Sr^{2+} and Ba^{2+} is observed. The effect of the crown ether is the most significant for Ba^{2+}, with the most rapid decrease in the Ba^{2+} mobility occurring when the concentration of the complexing reagent changes from 0 to 0.5 m*M*. Except for K^+, which shows a strong dependence on 18-crown-6 over the whole concentration range, the mobilities of Sr^{2+} and Ba^{2+} change less if more than 1 m*M* of 18-crown-6 is present in the BGE. The mobilities of the other cations are not significantly affected.

The effect of 18-crown-6 on the cations can be attributed to the fact that the oxygen of the reagent carries unshared pairs of electrons, through which cations are strongly solvated. This

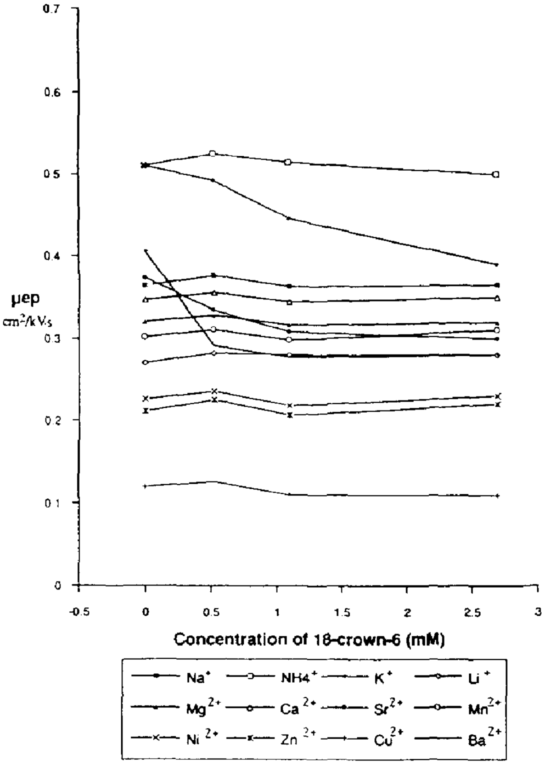

Fig. 6. Dependence of the ionic mobility μ_{ep} on the concentration of 18-crown-6. 2 μg/ml each of NH_4^+, K^+, Na^+, Li^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+} and Cu^{2+}. Experimental conditions: applied voltage, +20 kV; hydrostatic injection from 10 cm height for 30 s; BGE, 5 m*M* imidazole–6.5 m*M* HIBA–20% (v/v) methanol at pH 4.5.

leads to a new cation with a much larger size, which therefore moves more slowly than the bare cation in an electrical field. The stability of the newly formed cation depends on the fit between the ionic diameter of the cation and the cavity diameter of the crown ether. As reported [25], 18-crown-6 has a cavity diameter of 2.7 Å, which closely matches the ionic diameter of Ba^{2+}, 2.68 Å. Therefore Ba^{2+} can be tightly held by 18-crown-6. With a low concentration of 18-crown-6 present in the BGE, most of the Ba^{2+} is complexed. Therefore, its mobility changes only slightly when the concentration of 18-crown-6 is further increased. K^+ and Sr^{2+} have ionic diameters of 2.66 and 2.24 Å, respectively [26], and $\log \beta = 3.87$ for Ba^{2+}, 2.72 for Sr^{2+} and 2.03 for K^+ [27]. 18-Crown-6 was not found to produce a significant effect on the μ_{eof} because methanol is the predominant factor in determining μ_{eof}. This conclusion is based on a statistical estimation of main effects on the EOF. The concentrations of HIBA and 18-crown-6 and also methanol were changed simultaneously according to the 2^3 full factorial design [28]. The statistical results are given in Table 2. Only the methanol effect is significant at $\alpha = 0.05$. No interaction effects were found. This also agrees with the previous observation: as shown in Fig. 2, with more than 2 m*M* present in the BGE, the HIBA effect on μ_{eof} approaches a constant, whereas the effect of methanol on μ_{eof} is significant, as shown in Fig. 5.

3.4. Description of electrophoretic behaviour and prediction of the optimum separation

Using the preceding relationship, it is possible to find an optimum combination of the experimental parameters. As we have seen from

Table 2
Statistics for the evaluation of the main effects of μ_{eof}

Effect	Estimate	Mean square	Variance ratio
HIBA	−0.0018	0.0000	
Crown	0.0040	0.0000	
Methanol	−0.0287	0.0016	$27.28 > F_{0.05,1,4} = 7.7$[a]
HIBA/crown	−0.0046	0.0000	
HIBA/methanol	−0.0049	0.0000	
Crown/methanol	0.0024	0.0000	
HIBA/crown/methanol	−0.0084	0.0001	

[a] The value is larger than the critical *F* value, so that only the effect of methanol on μ_{eof} is statistically significant.

Fig. 6, the optimum separation conditions are located around 0.5 m*M* 18-crown-6 with 6.5 m*M* HIBA, 20% methanol and pH 4.5. An alternative approach is to apply experimental design and we wanted to investigate whether such an approach is feasible in this context and also if it would confirm our earlier findings.

In this set of experiments, dilute H_2SO_4 was replaced with dilute HCl for adjustment of the pH of the BGE because of the better solubility of chlorides than sulfates. Also, we intended to study the mobility dependence on HIBA without the presence of H_2SO_4 to confirm our previous explanation concerning the effect of HIBA and H_2SO_4.

A central composite design [28] was applied to select experimental points. The experimental setup was composed of fifteen experimental runs, in each of which two to five injections were performed to calculate the mean of the cation mobility. The concentration of HIBA varied from 2 to 6.5 m*M*, methanol from 10 to 20% and 18-crown-6 from 0.5 to 2.0 m*M*. The structure of the design is given in Table 3. For each cation, the effective mobility was calculated using the following equation:

$$\mu_{ep} = \frac{L_t L_d}{V}\left(\frac{1}{t} - \frac{1}{t_0}\right) \tag{2}$$

where L_t is the total length of the capillary, L_d the distance from the injector to the detector, V the applied voltage, t the migration time of the analyte cation and t_0 the migration time of water, which is used as the marker of EOF.

The modelling assumes a mathematical equation which relates the cation mobility (μ_{ep}) to the concentrations of HIBA (C_{HL}), 18-crown-6 (C_{crown}) and methanol ($C_{Me.}$):

$$\begin{aligned}\mu_{ep} &= k_0 + k_1 C_{HL} + k_2 C_{crown} + k_3 C_{Me.} \\ &\quad + k_{12} C_{HL} C_{crown} + k_{13} C_{HL} C_{Me.} \\ &\quad + k_{23} C_{crown} C_{Me.} + k_{11} C_{HL}^2 \\ &\quad + k_{22} C_{crown}^2 + k_{33} C_{Me.}^2\end{aligned} \tag{3}$$

where k represents the empirical parameter. The subsequent statistical evaluation by the SPSS program [17] gives the significant coefficients for individual cations,which are listed in Table 4. For all the cations, k_0 is significant. For NH_4^+, Li^+ and Na^+, only k_3 is significant. The fact that k_1 and k_2 are not significant is because there is no complexation between these cations and HIBA or 18-crown-6. For Mg^{2+}, Ca^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+}, k_1 and k_3 are significant. The coefficient k_{13} is never significant, indicating the absence of an interaction effect between HIBA and methanol. As we use HCl for electrolyte pH adjustment, a negative linear dependence of the mobilities of the alkaline earth and transition

Table 3
Central composite design for three factors at three levels

Run	Design matrix	HIBA (m*M*)	18-Crown-6 (m*M*)	Methanol (%, v/v)
1	0,0,0	4.25	1.25	15
2	1,1,1	5.85	1.78	18.5
3	−1,−1,−1	2.65	0.72	11.5
4	1,1,−1	5.85	1.78	11.5
5	−1,−1,1	2.65	0.72	18.5
6	1,−1,−1	5.85	0.72	11.5
7	−1,1,−1	2.65	1.78	11.5
8	1,−1,1	5.85	0.72	18.5
9	−1,1,1	2.65	1.78	18.5
10	1.414,0,0	6.5	1.25	15
11	−1.414,0,0	2	1.25	15
12	0,1.414,0	4.25	2	15
13	0,−1.414,0	4.25	0.5	15
14	0,0,1.414	4.25	1.25	20
15	0,0,−1.414	4.25	1.25	10

Table 4
Significant coefficients for individual cations in Eq. 4

Cation	k_0	k_1	k_2	k_3	k_{22}
NH_4^+	0.6332			−0.0073	
Li^+	0.3786			−0.0052	
Na^+	0.4696			−0.0055	
K^+	0.6437		−0.0371	−0.0088	
Sr^{2+}	0.5047		−0.0619	−0.0070	−0.0163
Ba^{2+}	0.4302		−0.0449	−0.0055	0.0149
Mg^{2+}	0.4860	−0.0059		−0.0063	
Ca^{2+}	0.5341	−0.0083		−0.0067	
Mn^{2+}	0.4906	−0.0082		−0.0067	
Ni^{2+}	0.4935	−0.0185		−0.0067	
Zn^{2+}	0.5013	−0.0212		−0.0069	

$\mu_{ep} = k_0 + k_1 C_{HL} + k_2 C_{crown} + k_3 C_{Me.} + k_{22} C_{crown}^2$. C_{HL} and C_{crown} are in m*M* and $C_{Me.}$ in % (v/v).

metal cations on the HIBA concentration is obtained. This is different from the results in Fig. 1 with H_2SO_4 which has been explained by the competition between HIBA and sulfate for the cations. Therefore, our previous explanation that sulfate, introduced by titration, serves as a complexing reagent has been confirmed by the experiments.

For K^+, Sr^{2+} and Ba^{2+}, k_2 and k_3 are significant and for the later two k_{22} is also important for a more accurate mobility prediction. Again, the interaction effects k_{12}, k_{13} and k_{23} are not significant. Therefore, Eq. 3 can be simplified to

$$\mu_{ep} = k_0 + k_1 C_{HL} + k_2 C_{crown} + k_3 C_{Me} + k_{22} C_{crown}^2 \qquad (4)$$

In most instances, a linear model is sufficient for the description of ionic mobility as a function of the system parameters. For Sr^{2+} and Ba^{2+}, an additional polynomial coefficient is included. The usefulness of the model for the optimization will be examined. The mobility of Cu^{2+} was not modelled because it moves much more slowly than the others and will not cause any problem in the separation.

The absolute value of $\Delta\mu_{min}$ was used as the criterion to search for the optimum experimental conditions. This is a more suitable criterion in our case than the α value (ratio of the migration times for two adjacent peaks) because peak cross-over is expected. Two neighbouring cations can be well resolved when their mobility difference is at least 0.005. Using the mobility equations given in Table 4, the mobility of each cation in the whole experimental domain is predicted. At each set of experimental conditions, the minimum mobility difference between each pair of cations was obtained, and consequently the maximum of the minimum within the whole experimental area was found. The maximum separation (maximum $\Delta\mu_{min}$) is always achieved at HIBA ≈ 6.5 m*M*. This means that the highest HIBA concentration should be used. However, as mentioned previously, the concentration is restricted. In the subsequent experiments, the HIBA concentration was set at 6.5 m*M*. Two optimum domains are obtained, which are shown in Fig. 7. Two sets of experimental conditions were selected from the two domains, namely HIBA = 6.5 m*M*, methanol = 20% and 18-crown-6 (a) 0.53 m*M* (found also by the preliminary experiments) or (b) 1.33 m*M*.

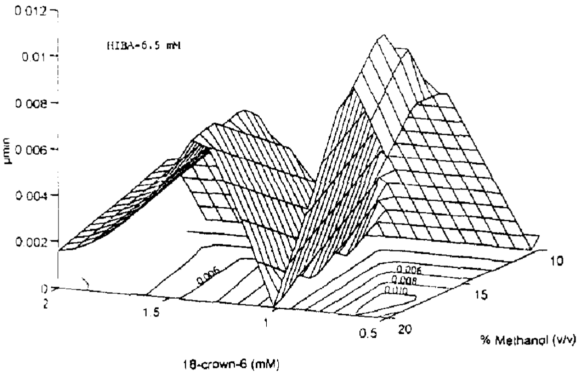

Fig. 7. Response surface for minimum differences in mobility ($\Delta\mu_{min}$).

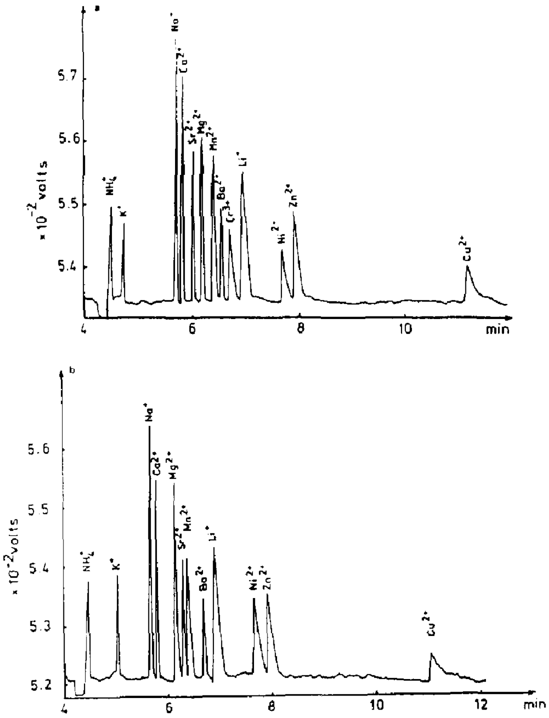

Fig. 8. Separation of a mixture containing NH_4^+, K^+, Na^+, Li^+, Mg^{2+}, Ca^{2+}, Cr^{3+} (2 μg/ml), Sr^{2+}, Ba^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+} (4 μg/ml) and Cu^{2+} (6 μg/ml). Experimental conditions: applied voltage, +20 kV; hydrostatic injection from 10 cm for 20 s; $I \approx 6.0$ μA; $T \approx 23$°C; BGE, 5 m*M* imidazole–6.5 m*M* HIBA–20% (v/v) methanol at pH 4.5 with (a) 0.53 and (b) 1.33 m*M* 18-crown-6.

Fig. 8a shows the separation of a mixture containing thirteen inorganic cations performed under the two sets of experimental conditions. Baseline separation for all the cations is achieved under conditions (a). One additional cation, Cr^{3+}, is also well resolved. Fig. 8b shows the separation performed under conditions (b). Simultaneous separation of the twelve cations is obtained. It is noted that sometimes the ammonium ion appears with the system peak, but this is not the case in the samples. A good linear relationship was obtained between the peak area and the concentration of ammonium ions. In this BGE system, ammonium ions do not interfere in the determination of potassium.

There is good agreement between the predicted and experimental mobilities as shown in Fig. 9.

3.5. Practical applicability of the selected BGE system

The practical applicability of the selected BGE was investigated by separating ions in different types of Chinese tea infusions under the optimum conditions. Good separation was achieved for all the samples. In Fig. 10a, an electropherogram of a green tea infusion is shown; five metal cations, K^+, Na^+, Ca^{2+}, Mg^{2+} and Mn^{2+}, are detected. Fig. 10b shows an electropherogram for the separation of ions in the tea sample spiked with ammonium nitrate, NH_4^+ and K^+ being well separated.

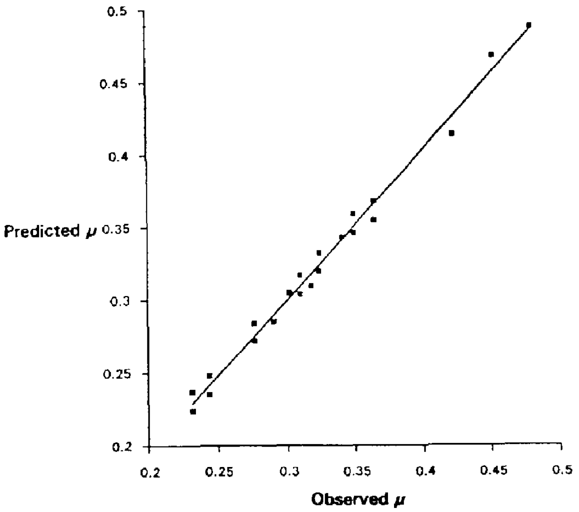

Fig. 9. Correlation plot of observed and predicted ionic mobilities of the cations under the two optimum experimental conditions (μ in $cm^2/kV \cdot s$). The correlation coefficients (R^2) is 0.992. The line represents the case of perfect correlation.

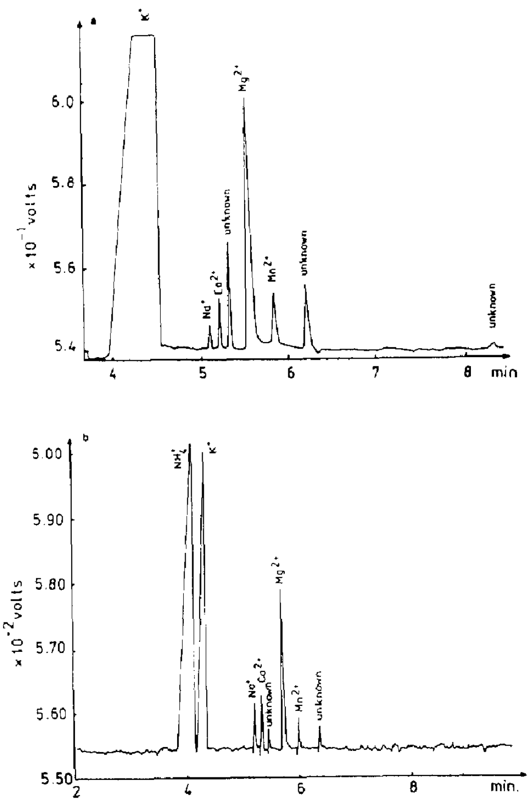

Fig. 10. Separation of Chinese green tea infusion. (a) Twofold dilution of green tea infusion; (b) 600-fold dilution of green tea infusion containing 1 $\mu g/ml$ of NH_4^+. Experimental conditions: applied voltage, +20 kV; electromigration injection, 30 kV for 10 s; $I \approx 6.0$ μA; $T \approx 23°C$; BGE, 5 m*M* imidazole–6.5 m*M* HIBA–20% (v/v) methanol–0.53 m*M* 18-crown-6 at pH 4.5.

3.6. Limit of detection

CZE separation is very efficient for a variety of substance types. The limit of detection attainable with the generally used UV detector is restricted, however. Sample injection by electromigration allows better results than hydrostatic injection. Therefore, we checked the limits of detection (LODs) with electromigration injection at 20 kV for 30 s. The LODs based on three times the baseline noise are as follows: 0.4 μg/l for Li^+ and Mg^{2+}, 1 μg/l for NH_4^+ and Ca^{2+}, 2.5 μg/l for Na^+, 10 μg/l for K^+, 120 μg/l for Sr^{2+}, Mn^{2+}, Cr^{3+} and Zn^{2+}, 500 μg/l for Ba^{2+} and Ni^{2+} and 1000 μg/l for Cu^{2+}.

As observed in our experiments, the improvement in detectability with electromigration injection has a greater impact on those cations which move faster. For a slowly moving cation such as Cu^{2+}, no significant effect was found.

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