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# Effect of the concentration of 18-crown-6 added to the electrolyte upon the separation of ammonium, alkali and alkaline-earth cations by capillary electrophoresis

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## Abstract

Capillary zone electrophoresis with indirect ultraviolet absorption detection was carried out to determine ammonium, alkali and alkaline-earth cations using a background electrolyte buffer containing 10 mM imidazole (pH 4.5) and 18-crown-6 as complexing agent. The optimization of the experimental parameters (18-crown-6 concentration, pH, temperature, running voltage) was studied. The crown ether concentration appeared to be a very convenient parameter to monitor the selectivity of the cations during the analysis of complex matrix aqueous samples. Adding 2.5 mM 18-crown-6 to the 10 mM imidazole electrolyte system (pH 4.5) generally gave the maximum resolution of ammonium and potassium cations; nevertheless, it was possible to shift the potassium peak in the electropherogram by adding up to 300 mM 18-crown-6. The stability constant of 18-crown-6 complexes with four cations ( $K^+$ ,  $NH_4^+$ ,  $Ba^{2+}$ ,  $Sr^{2+}$ ) has also been experimentally determined by capillary electrophoresis; it was confirmed that  $K^+$  has a stability complex constant with 18-crown-6 ( $\log K_s = 2.1$ ) higher than  $NH_4^+$  ( $\log K_s = 1.01$ ). Simultaneous quantitation of sodium, calcium, potassium, ammonium, magnesium and lithium has been performed in such optimized separation conditions.

## 1. Introduction

Capillary electrophoresis (CE) is currently undergoing rapid development owing to its efficiency, high resolution, relative simplicity, UV detection at low wavelengths (190 nm), speed and automatization of separations, and low buffer consumption. Its drawbacks compared to liquid-phase chromatography are the rather weak reproducibility of the electroosmotic flow assuring the migration times, and a lower con-

centration sensitivity. However, this technique is available not only for the separation of inorganic and organic anions and cations, but also for the separation of ionizable and neutral organic molecules.

During the last five years, CE has been widely applied to the analysis of inorganic cations in various aqueous samples [1–16]; the analytical approach is based on the indirect detection mode because of the transparency of inorganic cations in the UV region, and separation using operating conditions such as the electroosmotic flow must be in the same direction as the electrophoretic

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mobility of the ions analyzed in order to minimize their migration time.

Initially, Aguilar et al. [2] separated the cations  $\text{Fe}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  in the form of their cyanide complexes in CE; a phosphate buffer (20 mM) was used at pH 7 and direct UV detection was carried out at 214 nm. Then, Swaile and Sepaniak [11] used a laser-based fluorimetric detector to detect cations  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$ , complexed by 8-hydroxyquinoline-5-sulfonic acid; this technique was applied to the detection of calcium(II) and magnesium(II) in blood serum.

Foret et al. [4] separated fourteen lanthanides by CE using an electrophoretic buffer consisting of an  $\alpha$ -hydroxyisobutyric acid as complexing agent and creatinine as the indirect UV detector marker; in fact, the similarity between the electrophoretic mobilities of alkali, alkaline-earth, rare-earth and metal cations requires the addition of a water-soluble complexing reagent to the electrolyte. The electrophoretic mobility of each cation thus decreases following their complexation in situ by  $\alpha$ -hydroxyisobutyric acid, and selectivity is greatly improved. The factors affecting the separation of such cations were studied by Weston and co-workers [5,6]; the complexing agent was also  $\alpha$ -hydroxyisobutyric acid and the compound allowing indirect UV detection was called UV Cat 1, a proprietary reagent developed at Waters. Very recently, Beck and Engelhardt [8] firstly proposed a nice alternative way for the separation of several alkali and alkaline-earth cations by using an imidazole-based electrophoretic buffer and indirect UV detection at 214 nm; their results demonstrated the applicability of this running electrolyte in routine analysis. Riviello and Harold [15] reported another electrolyte system based on copper(II) as the primary electrolyte constituent; the determination of alkali metals, alkaline-earth metals and ammonium was obtained in less than 5 min. Finally, CE has recently received a great deal of attention as a tool for the analysis of transition metal cation mixtures [17–22]. Thus, Shi and Fritz [17] reported the separation of sixteen common metal ions and ammonium at pH 4.3, using 4-methyl-

benzylamine as a reagent for indirect detection, and lactic acid and 18-crown-6 as complexing agents.

However, potassium and ammonium cations have nearly identical ionic mobilities and thus comigrate under acidic pH conditions; the most common alternative is to add 18-crown-6 to the electrolyte in order to promote complex formation with these two cations. However, the concentration of 18-crown-6 added to the imidazole electrolyte also influences the separation performances (electrophoretic mobility, peak efficiency, asymmetry factor and resolution of the other analyte cations).

The UV-absorbing organic cation (imidazolium), which has a quite similar electrophoretic mobility to that of these inorganic cations, was selected as the main constituent of the buffer, and 18-crown-6 was added in the electrolyte as the complexing agent. So, we report the role of 18-crown-6 added to the imidazole electrolyte for analyzing  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Li}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{NH}_4^+$  cation mixtures by CE. Then, a method is presented to determine the stability constant of 18-crown-6 complexes with these cations.

## 2. Experimental

### 2.1. Apparatus

Separations were carried out on a P/ACE 2100 and P/ACE 5000 apparatus (Beckman Instruments, Fullerton, CA, USA) equipped with a UV detector with wavelength filters of 190, 200, 214, 254, 260 and 280 nm. Fused-silica capillaries (Beckman Instruments) 57 cm (50 cm to the detector)  $\times$  75  $\mu\text{m}$  I.D.  $\times$  375  $\mu\text{m}$  O.D. were used. The part of the capillary where separation takes place was kept at a constant temperature (25°C) by immersion in a cooling liquid circulating in the cartridge with a detection aperture of 100  $\mu\text{m}$   $\times$  800  $\mu\text{m}$ . The solutes were injected at the anode end of the capillary in the hydrodynamic mode by azote superpressure (0.5 p.s.i). Data were collected using a IWT data acquisition system. The detector time constant

was 0.1 s and the data acquisition rate was 20 Hz.

The pH of each solution was verified on a Model  $\phi 10$  pH meter (Beckman, Fullerton, CA, USA). The capillary tubing of fused silica was conditioned daily by rinsing with a solution of 1 M sodium hydroxide (10 min), then water (10 min), and finally the electrophoretic buffer (15 min). Between two consecutive analyses, the capillary was rinsed with water (3 min) and then with the electrophoretic buffer (5 min) to improve the reproducibility of the electroosmotic flow and migration times of the solutions. Linear regression were made using Regrelis program (Logedic, Vesoul, France).

## 2.2. Chemicals

All chemical products used were of analytical quality. Imidazole (99% purity) was obtained from Sigma (St. Louis, MO, USA) and 18-crown-6 from Aldrich (Milwaukee, WI, USA). The water used in the preparation of buffers and the water necessary for dilutions was of HPLC quality (Fisons, Farmitalia, Milan, Italy). The running buffer contained 10 mM imidazole and the concentration of 18-crown-6 varied from 0.01 up to 300 mM; the electrophoretic buffer pH was adjusted to the desired value by adding a 1 M stock solution of acetic acid (Carlo Erba, Milan, Italy). Finally, each buffer or rinsing solution was filtered before use through a membrane filter having a diameter of 25 mm and porosity of 0.2  $\mu\text{m}$  (Whatman, Maidstone, UK).

## 2.3. Electrophoretic mobility determination procedure

From experimental data, the electrophoretic mobility ( $m^+$ ) of a cation  $C^+$  can be determined by

$$m^+ = \frac{L_d L_t}{V} \left( \frac{1}{t_m} - \frac{1}{t_0} \right) \quad (1)$$

where  $L_d$  is the length of the capillary from the inlet to the detector,  $L_t$  is the total length of the capillary,  $V$  the applied voltage,  $t_m$  the migration

time of the cation and  $t_0$  the migration time of a neutral marker (water).

## 3. Results and discussion

The use of indirect UV absorption is required since alkali and alkaline-earth metals have no UV absorbance. Hence, the electrophoretic buffer used for the separation of inorganic cations must contain an organic cation whose electrophoretic mobility must closely match those of the analyte cations, in order to obtain highly symmetrical peak shapes, and which also has an intense chromophore group in the UV region. As initially proposed by Beck and Engelhardt [8] imidazole (or 1,3-diaza-2,4-cyclopentadienyl ring) is a heterocyclic azote ( $\text{p}K_{a_1} = 6.9$  and  $\text{p}K_{a_2} = 14.5$ ) whose UV absorption spectrum has a maximum at 211 nm (pH 4.5). Besides, the imidazolium cation appears to have an electrophoretic mobility ( $45.8 \cdot 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$  determined in 10 mM sodium acetate at pH 4.5) close to those of magnesium ( $46.3 \cdot 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ ) and sodium ( $48.0 \cdot 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ ), both determined in 10 mM imidazole electrolyte (pH 4.5). The sample injection was thus carried out at the anodic end of the capillary and detection at the cathodic end; the electroosmotic flow was in the same direction as the electrophoretic mobility of the cation.

The simultaneous determination of potassium and ammonium is important in agricultural, food and water samples. As potassium and ammonium have nearly identical electrophoretic mobilities, they would comigrate in the 10 mM imidazole electrolyte (pH 4.5). Two alternatives may exist. (i) Using an alkaline solution of benzylamine (10 mM, pH 9.25) as carrier electrolyte which allows the separation of potassium and ammonium cations by decreasing the electrophoretic mobility of the ammonium cation [1,6,14]; thus, the potassium cation migrates faster than ammonium but the electroosmotic flow increases, bringing about a shorter analysis time. Consequently, we cannot resolve all inorganic cations in a single run [14]. (ii) Adding

18-crown-6 to the electrolyte in order to promote complex formation with these two cations. The addition of this weak complexing agent alters the electrophoretic mobilities of the cations and their relative selectivity would depend on 18-crown-6 concentration. An inorganic cation ( $C^+$ ) interacts with the neutral 18-crown-6 (L) according to the following chemical equilibrium:



where  $K_s$  is the stability complex constant of the complex  $CL^+$  between  $C^+$  and the ligand L. The electrophoretic mobility ( $m^+$ ) of the cation  $C^+$  was determined as the weighted average of the electrophoretic mobility of the free cation ( $m_{C^+}$ ) and of the complexed cation ( $m_{CL^+}$ ) with 18-crown-6, as expressed by the following relationship:

$$m^+ = \frac{[C^+]}{[C^+] + [CL^+]} m_{C^+} + \frac{[CL^+]}{[C^+] + [CL^+]} m_{CL^+} \quad (3)$$

where  $[C^+]$  and  $[CL^+]$  represent the concentration at equilibrium of the free and complexed cation  $C^+$  and  $CL^+$ , respectively. At a given pH value, the stability constant  $K_s$  of the complex  $CL^+$  can be expressed as:

$$K_s = \frac{[CL^+]}{[C^+][L]} \quad (4)$$

where  $[L]$  is the 18-crown-6 concentration at equilibrium.

Combining eqs. 3 and 4 gives the following expression for the calculation of  $m^+$ :

$$m^+ = \frac{1}{1 + K_s[L]} m_{C^+} + \frac{K_s[L]}{1 + K_s[L]} m_{CL^+} \quad (5)$$

According to Eq. 5, the variation of the electrophoretic mobility ( $m^+$ ) versus the value of the negative logarithm of the 18-crown-6 concentration ( $pL = -\log [L]$ ) allows to determine the stability constant  $K_s$ ; indeed, this curve has a shape similar to an acid–base titration curve, and the abscissa of the inflection point will be equal to  $\log K_s$ .

### 3.1. Effect of 18-crown-6 concentration on separation performances

The effect of imidazole concentration on selectivity was studied in the 1–15 mM range, by maintaining a constant pH value of 4.5. A significant modification in selectivity was observed for sodium and magnesium cations when the imidazole concentration varied from 1 up to 15 mM (Fig. 1a). While calcium and sodium cations are always well resolved whatever the imidazole concentration, the background co-ion concentration needs to be carefully selected in order to avoid comigration of sodium and magnesium (3 mM). Thus, sodium migrates faster than magnesium at imidazole concentrations higher than 3 mM, while a reversed migration order occurs at lower concentrations, which may be explained by a possible complexation of these cations with imidazole. Migration times decrease with the equivalent conductance limit, according to the following decreasing migration time order for the cations: potassium ( $\lambda_{K^+} = 73.5 \text{ S cm}^2$ ), calcium ( $\lambda_{Ca^{2+}} = 59.5$ ), magnesium ( $\lambda_{Mg^{2+}} = 53.1$ ), sodium ( $\lambda_{Na^+} = 50.1$ ) and finally lithium ( $\lambda_{Li^+} = 38.7$ ). Our migration order is not identical to that reported by Beck and Engelhardt [8], but the running electrolyte has not exactly the same composition and it is well known that the co-ion buffer concentration may influence the migration of the analytes.

Thus, if alkali and alkaline-earth cations are resolved by using an imidazole running electrolyte, potassium and ammonium cations would comigrate under non-complexing conditions. The addition of a complexing agent (18-crown-6) to the electrolyte is necessary for the total resolution of these two cations. The concentration of 18-crown-6 added to the imidazole electrolyte may influence the separation performances (electrophoretic mobility, peak efficiency, asymmetry factor and resolution of the other analyte cations). Besides, no complete study has been previously performed on the selectivity change due to a modification of this complexing agent concentration in the imidazole electrolyte. So, electroosmotic mobility and electrophoretic mobilities of six cations have been determined over

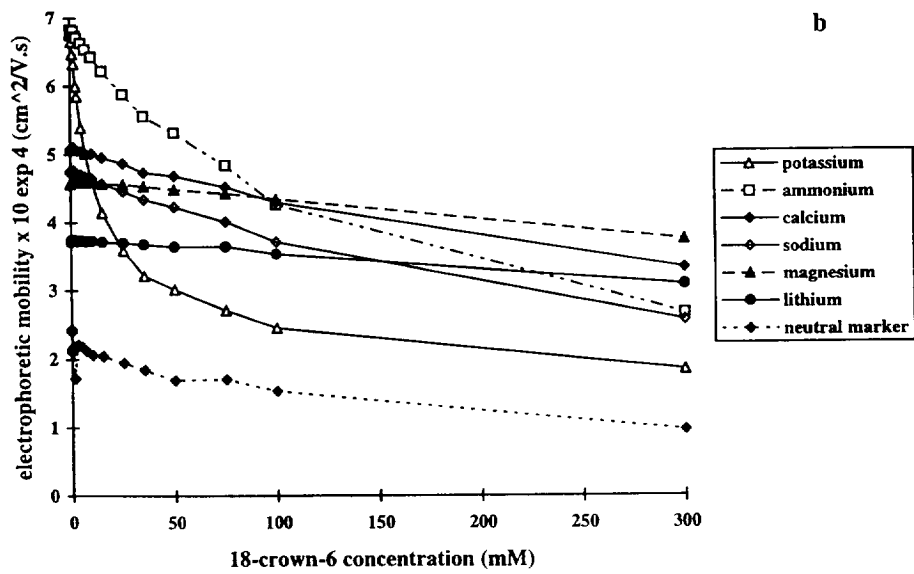
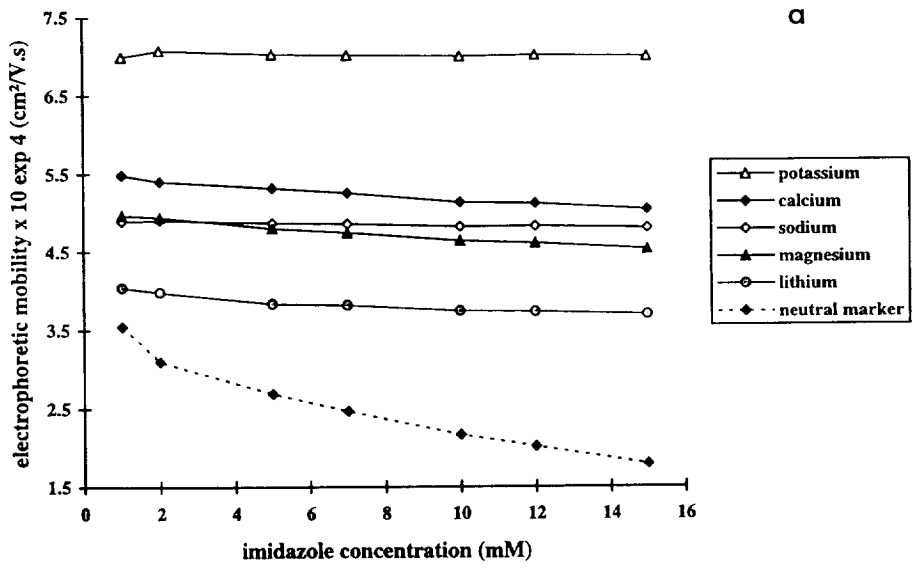


Fig. 1.

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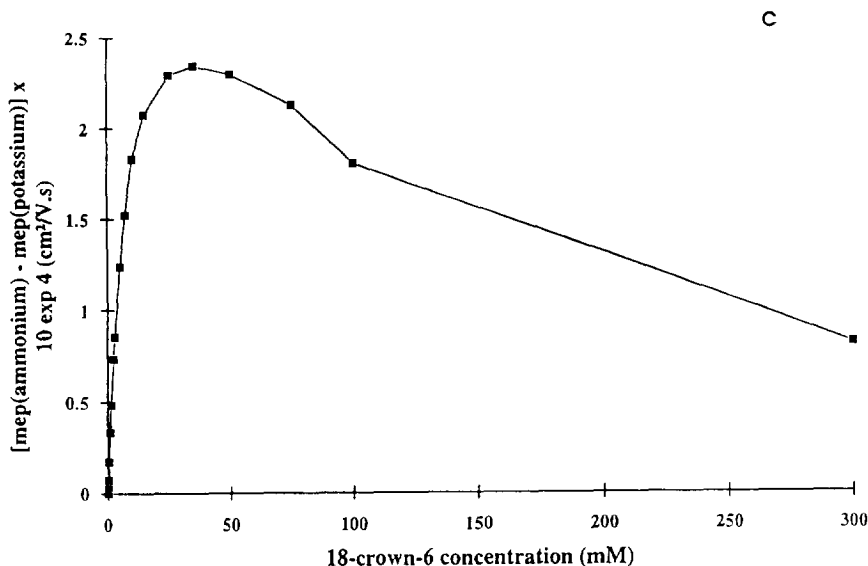


Fig. 1. (a) Influence of imidazole concentration on the electrophoretic mobility of several cations. Fused-silica capillary dimensions, 57 cm (50 cm to detector)  $\times$  75  $\mu$ m I.D., 100  $\mu$ m  $\times$  800  $\mu$ m aperture; electrolyte, imidazole, pH 4.5; indirect UV detection, 214 nm; applied voltage, 15 kV (positive polarity); temperature, 25°C; hydrodynamic injection, 2 s; cation concentration, 5 ppm. (b) Influence of 18-crown-6 concentration added to the imidazole running electrolyte on the electrophoretic mobility of several cations. Experimental conditions as in (a) except electrolyte (10 mM imidazole, 18-crown-6, pH 4.5) and applied voltage (30 kV). (c) Difference in electrophoretic mobilities of ammonium and potassium versus the concentration of 18-crown-6. Experimental conditions as in (b).

the  $10^{-5}$ – $3 \cdot 10^{-1}$  M 18-crown-6 concentration range using 10 mM imidazole buffer at pH 4.5 (Table 1). By increasing the 18-crown-6 concentration in the running electrolyte (10 mM imidazole, pH 4.5), the electrophoretic mobilities of potassium and ammonium decrease more rapidly than the electrophoretic mobilities of other cations (Fig. 1b); up to 7 mM, the migration order of these cations is not modified while a reversal of migration order of calcium and potassium is observed beyond this concentration. According to a previous study [16], the crown-complexed cations have lower ionic mobilities than the solvated cations. Besides, several migration order and selectivity changes are also observed for other cations at higher complexing agent concentration: for example, potassium migrates more slowly than magnesium beyond 10 mM, then more slowly than lithium beyond 25 mM. Fig. 2 illustrates the effect of 18-crown-6 concentration on the separation of a standard mixture of six cations. It is possible to shift the potassium peak in the electropherogram by add-

ing a selected 18-crown-6 concentration to the 10 mM imidazole electrolyte (pH 4.5): so, potassium matrix samples will be resolved by using 35 mM 18-crown-6 in the electrolyte while ammonium matrix samples will be better resolved beyond 300 mM 18-crown-6. We have calculated the difference in electrophoretic mobilities of ammonium and potassium versus the concentration of 18-crown-6; the selectivity between these two cations does not remain constant and reaches a maximum at 35 mM (Fig. 1c), unlike previously reported results [12]. So, the crown ether concentration appears to be a very convenient parameter to monitor the selectivity of cations, particularly during the analysis of potassium and sodium matrix samples.

Finally, the variation of crown ether concentration also allows the determination of the stability complex constant of each cation by plotting the electrophoretic mobility versus the mathematical expression:  $-\log[18\text{-crown-6}]$  (Fig. 3a). The abscissa of the inflection point is equal to  $\log K_s$ , and the stability complex constants

Table 1  
Influence of 18-crown-6 concentration on electrophoretic mobility ( $m_{ep}$ ) of some inorganic cations

18-Crown-6 concentration (mM)	$m_{ep} \times 10^{-4}$ (cm <sup>2</sup> /V s)					
	Potassium	Ammonium	Calcium	Sodium	Magnesium	Lithium
0.01	6.768	6.768	5.057	4.732	4.566	3.708
0.02	6.773	6.773	5.063	4.737	4.572	3.714
0.05	6.773	6.773	5.061	4.737	4.572	3.712
0.07	6.810	6.810	5.085	4.758	4.592	3.726
0.1	6.775	6.812	5.077	4.754	4.592	3.732
0.2	6.755	6.826	5.089	4.748	4.599	3.736
0.5	6.655	6.828	5.089	4.759	4.599	3.729
1	6.475	6.808	5.086	4.758	4.601	3.735
1.5	6.331	6.815	5.106	4.768	4.621	3.745
2.5	5.999	6.733	5.069	4.734	4.596	3.734
3	5.852	6.706	5.059	4.723	4.590	3.725
5	5.392	6.630	5.049	4.706	4.597	3.731
7	5.016	6.538	5.016	4.676	4.588	3.724
10	4.597	6.430	5.012	4.650	4.597	3.728
15	4.145	6.221	4.952	4.570	4.570	3.715
25	3.589	5.882	4.871	4.461	4.561	3.701
35	3.216	5.559	4.733	4.338	4.530	3.677
50	3.018	5.315	4.685	4.230	4.486	3.646
75	2.710	4.837	4.520	4.011	4.426	3.646
100	2.454	4.262	4.295	3.715	4.341	3.534
300	1.851	2.670	3.333	2.572	3.751	3.088

Experimental conditions as in Fig. 1b.

with 18-crown-6 have been determined at 25°C for potassium, ammonium, barium and strontium (Table 2); these values agree with those reported in the literature [23,24]. The high  $K_s$  value for potassium results in a migration speed that is reduced relative to ammonium, enabling the two cations to be separated. On the other hand, the weaker value of  $K_s$  for sodium, calcium, magnesium and lithium explain the flat shape of the curves (Fig. 1b).

However, the complexation process may be easily followed by the degree of complexation  $x$  of the cation  $C^+$ , defined as

$$x = \frac{[CL^+]}{[C^+] + [CL^+]} = \frac{m_{C^+} - m^+}{m_{C^+} - m_{CL^+}} \quad (6)$$

where  $m_{CL^+}$ ,  $m_{C^+}$  and  $m^+$  are the electrophoretic mobilities of the complexed cation, the free cation and the partially complexed cation by the 18-crown-6, respectively. The variation of com-

plexation degree versus the expression  $-\log[18\text{-crown-6}]$  is shown in Fig. 3b.

In order to determine the best separation conditions, a series of 10 mM imidazole running electrolytes having different 18-crown-6 concentrations (0.1–8 mM) were tested. Fig. 4 reports the change in resolution between two consecutive peaks. In particular, the resolution between ammonium and potassium peaks and also between potassium and calcium peaks are very sensitive to complexing agent concentration. However, a modification of 18-crown-6 concentration does not improve the resolution between the other cations. Overall, running electrolyte with 2.5 mM 18-crown-6 seems to give the best total resolution.

### 3.2. Effect of running electrolyte pH

The influence of running electrolyte pH upon electrophoretic mobility and resolution of several

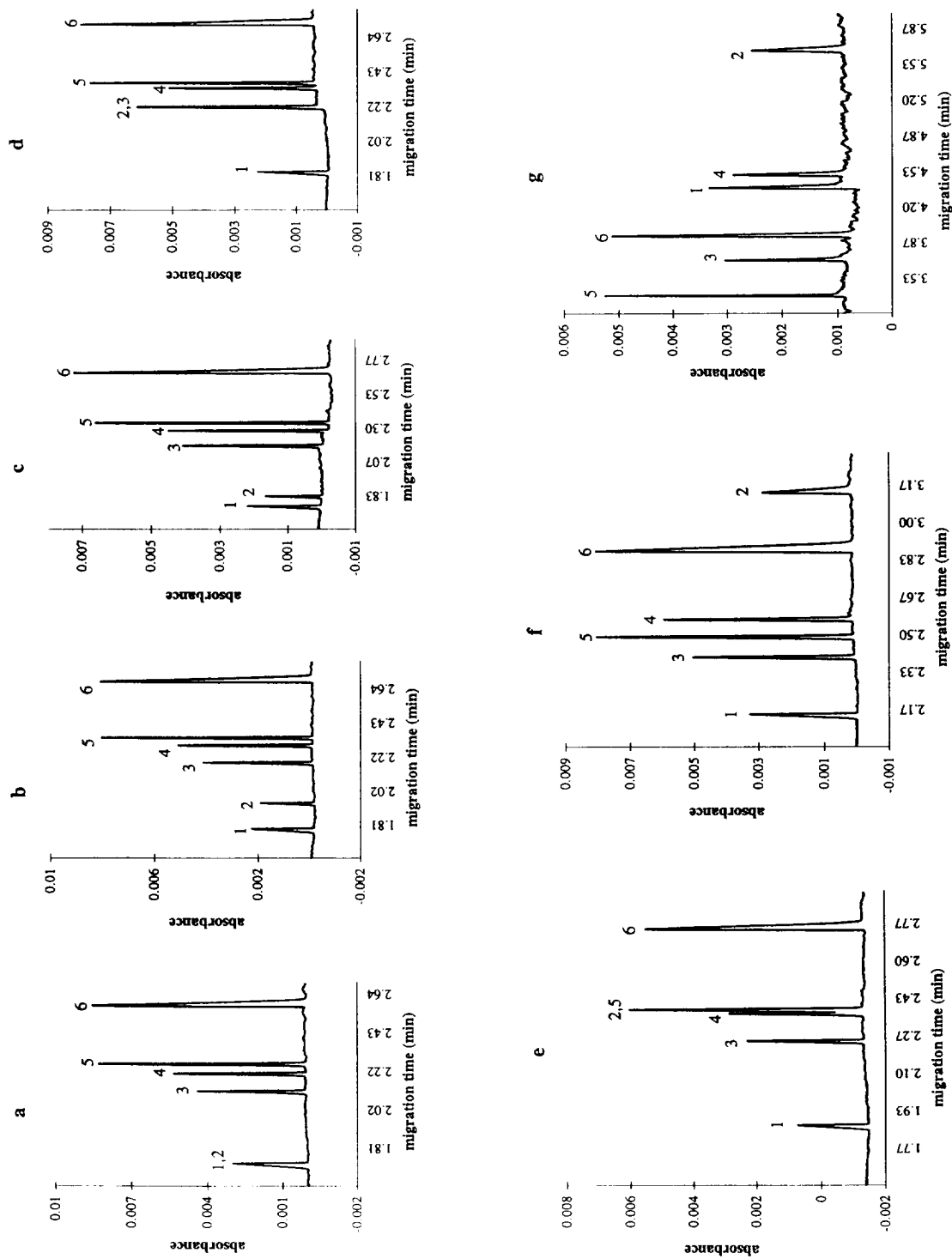


Fig. 2. Influence of 18-crown-6 concentration added to the imidazole running electrolyte on the migration order of an inorganic cation mixture. Experimental conditions as in Fig. 1b except 18-crown-6 concentration: (a) 0.01 mM; (b) 1.0 mM; (c) 2.5 mM; (d) 7.0 mM; (e) 10 mM; (f) 300 mM. Cations (5 ppm): 1 =  $\text{NH}_4^+$ ; 2 =  $\text{K}^+$ ; 3 =  $\text{Ca}^{2+}$ ; 4 =  $\text{Na}^+$ ; 5 =  $\text{Mg}^{2+}$ ; 6 =  $\text{Li}^+$ .



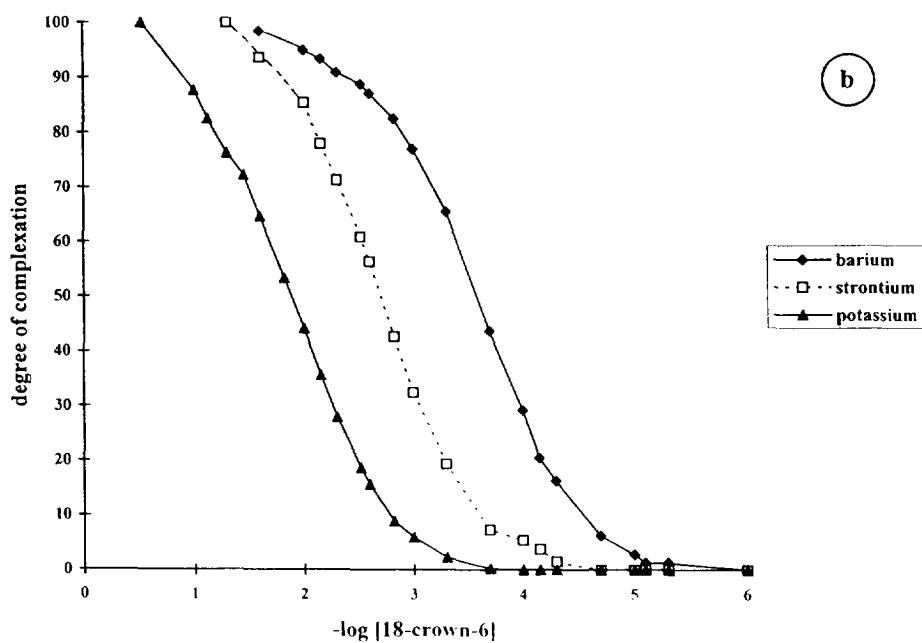
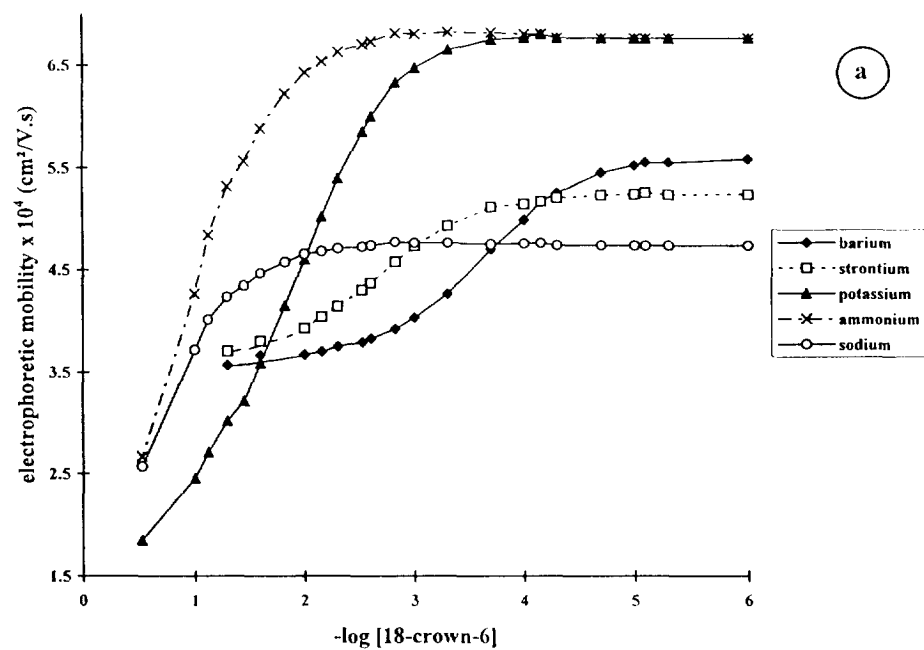


Fig. 3. Variation of the electrophoretic mobility and complexation degree of several inorganic cations versus the mathematical expression:  $-\log [18\text{-crown-6}]$ . Experimental conditions as in Fig. 1b.

Table 2  
Stability constant of 18-crown-6 complexes for several cations

Cation	$\log K_{\text{exp}}$	$\log K^{\text{a}}$	$\log K^{\text{b}}$
Ammonium	1.01	0.9	1.23
Potassium	2.1	2.1	2.03
Barium	3.6	4.1	3.87
Strontium	2.7	2.9	2.72
Calcium	–	1.0	–
Sodium	–	1.0	0.8

Experimental conditions as in Fig. 1b.

<sup>a</sup> Literature data were taken from Ref. [24].

<sup>b</sup> Literature data were taken from Ref. [23].

cations has been performed on imidazole electrolyte without addition of 18-crown-6, then with addition of 2.5 mM 18-crown-6 (Fig. 5). The electroosmotic flow increased as the pH increased from 3.5 to 6.0, hence the CE separation time could be shortened at pH 6.0. The complex

formation between 18-crown-6 and cations was obviously independent of the pH of the running electrolyte (Fig. 5a and c). We also found that the resolution was not very sensitive to pH using imidazole electrolyte (Fig. 5b) whereas the resolution between  $\text{K}^+$  and  $\text{Ca}^{2+}$  or  $\text{Ca}^{2+}$  and  $\text{Na}^+$  depended on the pH using this complexing electrolyte (Fig. 5d).

### 3.3. Effect of running voltage

The effect of voltage on separation resolution has been studied using imidazole- and 18-crown-6-based electrolyte. An increasing voltage between 5 and 30 kV induced a shorter analysis time but a constant electrophoretic mobility of cations (Fig. 6). Moreover, an increasing voltage induced an increase in peak efficiency and resolution (Table 3). Fig. 6 shows that the current linearly increases with applied voltage; therefore, the amount of heat generated is totally

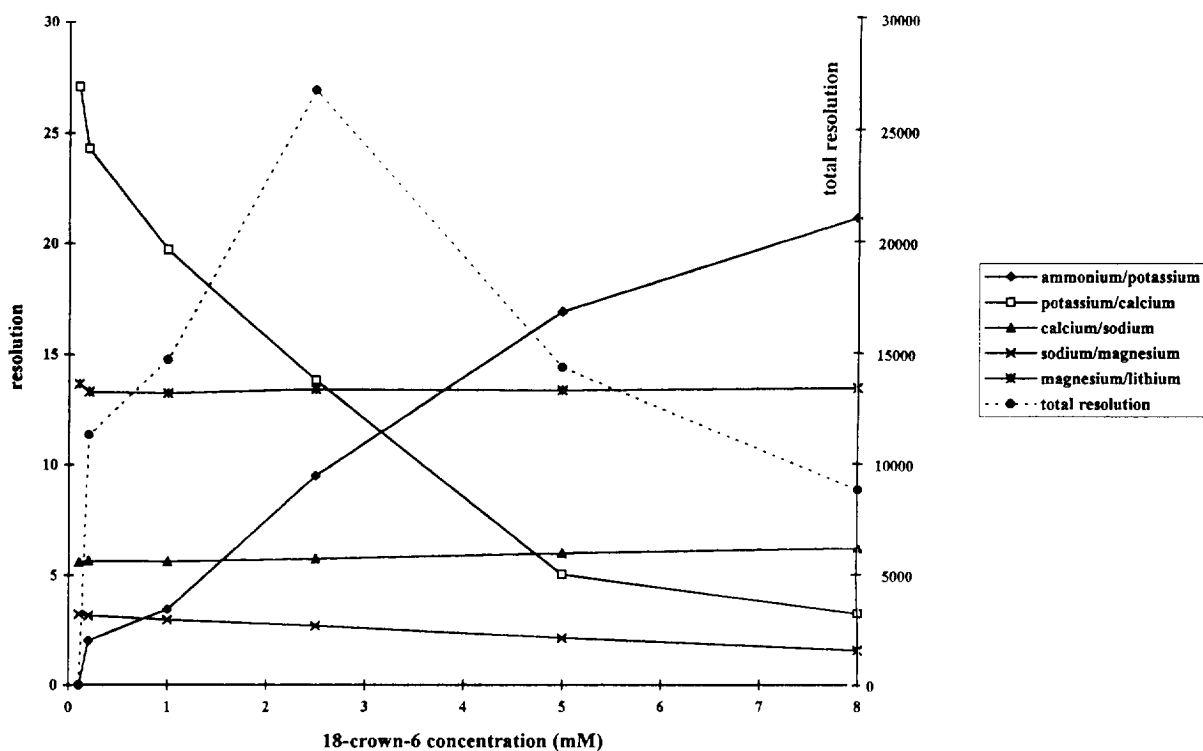


Fig. 4. Influence of 18-crown-6 concentration on the CE resolution of several cations. Experimental conditions as in Fig. 1b.

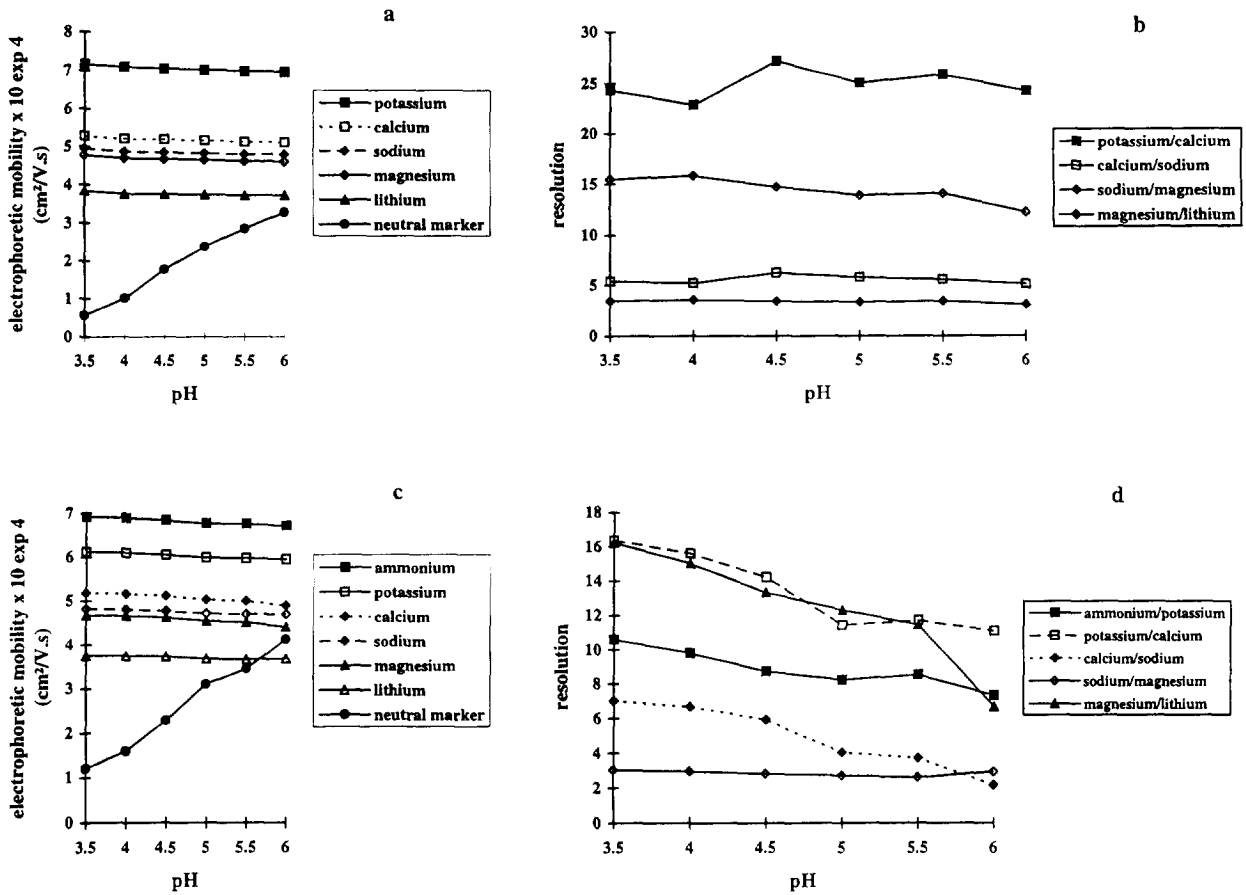


Fig. 5. Influence of buffer pH on the electrophoretic mobility and resolution of several cations. Fused-silica capillary dimensions, 57 cm (50 cm to detector)  $\times$  75  $\mu$ m I.D., 100  $\mu$ m  $\times$  800  $\mu$ m aperture; electrolyte, 10 mM imidazole; indirect UV detection, 214 nm; applied voltage, 20 kV (positive polarity); hydrodynamic injection, 2 s; cation concentration, 5 ppm. (a, b) without 18-crown-6; (c, d) with 2.5 mM 18-crown-6.

dissipated. Thus, we can work equally well at 5 as at 30 kV. The 75  $\mu$ m I.D. of the capillary tube allows a good thermal dispersion.

### 3.4. Effect of temperature

Temperature variations affect several physical parameters (viscosity, dielectric constant, pH) and consequently the electroosmotic flow-rate and the electrophoretic mobility of the analytes. This study was carried out at five different temperatures (20, 25, 30, 35, 40°C) with an imidazole- and 18-crown-6-based electrolyte. Temperature variations in the range 20–40°C did not significantly alter the selectivity of our separa-

tion at pH 4.5. When the temperature increased from 20 to 40°C, the migration times decreased and, thus, the electrophoretic mobilities of cations increased (Fig. 7). The peak efficiency was slightly better at low temperatures because the diffusion coefficient decreased at lower temperatures; thus, working at 25°C maintains a high peak efficiency and resolution without too long analysis times (Table 4).

### 3.5. Quantitative aspect

The influence of hydrodynamic injection time on the corrected peak area, peak area and peak height was studied with an imidazole and 18-

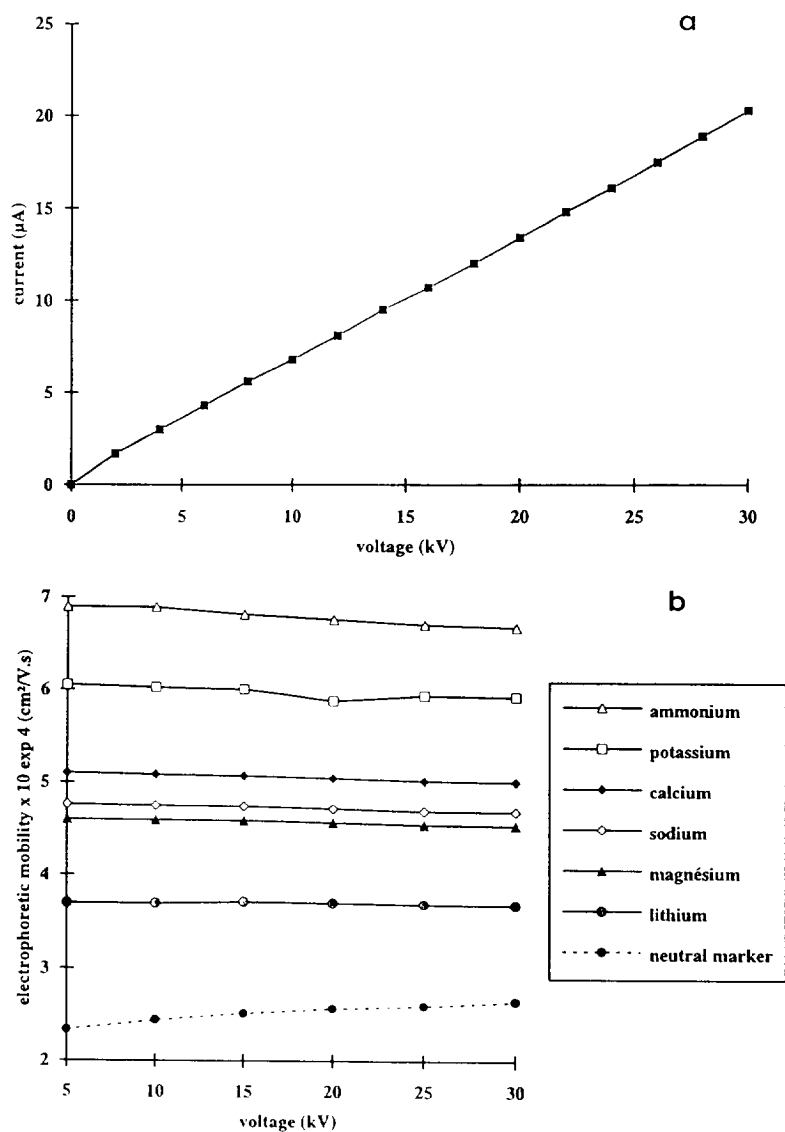


Fig. 6. Dependence of cation electrophoretic mobility and current versus running voltage. Experimental conditions as in Fig. 5 except electrolyte (10 mM imidazole, 2.5 mM 18-crown-6, pH 4.5). (a) Current; (b) electrophoretic mobility.

crown-6-based electrolyte at pH 4.5. Fig. 8a–c shows that it is preferable to work with corrected peak area, where the correlation coefficients are slightly higher than those obtained with peak area (Table 5). On the other hand, when the injection time increases from 1 to 10 s, peak height is not a linear function, except for sodium and magnésium. Indeed, these two cations have an electrophoretic mobility close to that of

imidazole ( $4.709 \cdot 10^{-4}$ ,  $4.562 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$  in an imidazole and 18-crown-6-based electrolyte and  $4.583 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$  in an acetate electrolyte, respectively). So, if the electrophoretic mobility of the cation is close to that of the co-ion and if the limited column capacity is not reached, it is possible to use the peak height for quantitation. In our case, the correlation coefficient for sodium and magnésium are 0.99932 and

Table 3  
Influence of applied voltage on peak efficiency ( $N$ ) and resolution ( $R_s$ )

Voltage (kV)	$N$ (ammonium)	$N$ (potassium)	$N$ (calcium)	$N$ (sodium)	$N$ (magnesium)	$N$ (lithium)
5	71 800	104 500	172 900	113 800	191 900	78 800
10	89 500	179 400	278 800	213 100	320 100	109 900
15	80 900	199 500	277 400	262 700	368 100	89 400
20	82 000	209 700	295 500	302 100	380 800	89 200
25	99 100	228 800	290 500	340 800	371 600	94 200
30	101 600	280 400	296 400	348 600	374 200	113 500
	$R_s$ (ammonium–potassium)	$R_s$ (potassium–calcium)	$R_s$ (calcium–sodium)	$R_s$ (sodium–magnesium)	$R_s$ (magnesium–lithium)	
5	7.07	11	4.36	2.18	11.73	
10	8.2	13.89	5.66	2.82	13.96	
15	7.9	14.07	5.84	2.97	12.85	
20	7.82	14.31	6.05	3.05	12.71	
25	8.19	14.47	6.14	3.06	12.76	
30	8.37	15.17	6.12	3.04	13.54	

Experimental conditions as in Fig. 6.

0.99955, respectively. The repeatability in terms of the migration times was less than 0.5% R.S.D. and, in terms of corrected peak area, was better than 5% R.S.D.

A notable decrease in efficiency was observed when the injection time increased from 1 to 10 s. For example, there was 81% decrease in peak efficiency for ammonium and 80% for lithium for a concentration of 2 ppm. This loss of peak efficiency was also less important for the cations

having an electrophoretic mobility close to that of imidazole (15% decrease). This study confirms the limited column capacity in free capillary electrophoresis.

The influence of solute concentration on the three parameters was studied with an imidazole and 18-crown-6-based electrolyte at pH 4.5 Fig. 9a–c shows the influence of cation concentration in the 2.5–50 ppm range on the corrected peak area, peak area and peak height. The correlation

Table 4  
Influence of temperature on peak efficiency ( $N$ ) and resolution ( $R_s$ )

Temperature (°C)	$N$ (ammonium)	$N$ (potassium)	$N$ (calcium)	$N$ (sodium)	$N$ (magnesium)	$N$ (lithium)
20	124 600	350 500	436 900	469 700	557 800	146 300
25	110 510	305 700	362 700	463 800	561 800	107 900
30	93 100	218 200	316 500	378 800	439 100	93 900
35	104 300	231 600	332 000	393 300	410 700	124 600
40	92 700	196 300	322 100	341 600	381 900	107 400
	$R_s$ (ammonium–potassium)	$R_s$ (potassium–calcium)	$R_s$ (calcium–sodium)	$R_s$ (sodium–magnesium)	$R_s$ (magnesium–lithium)	
20	12.02	17.26	7.81	3.9	16.6	
25	8.7	16.37	6.98	3.47	14.21	
30	6.51	14.86	6.08	2.76	12.39	
35	5.91	15.59	5.93	2.55	13.22	
40	4.79	14.97	5.4	2.28	12.07	

Experimental conditions as in Fig. 7.

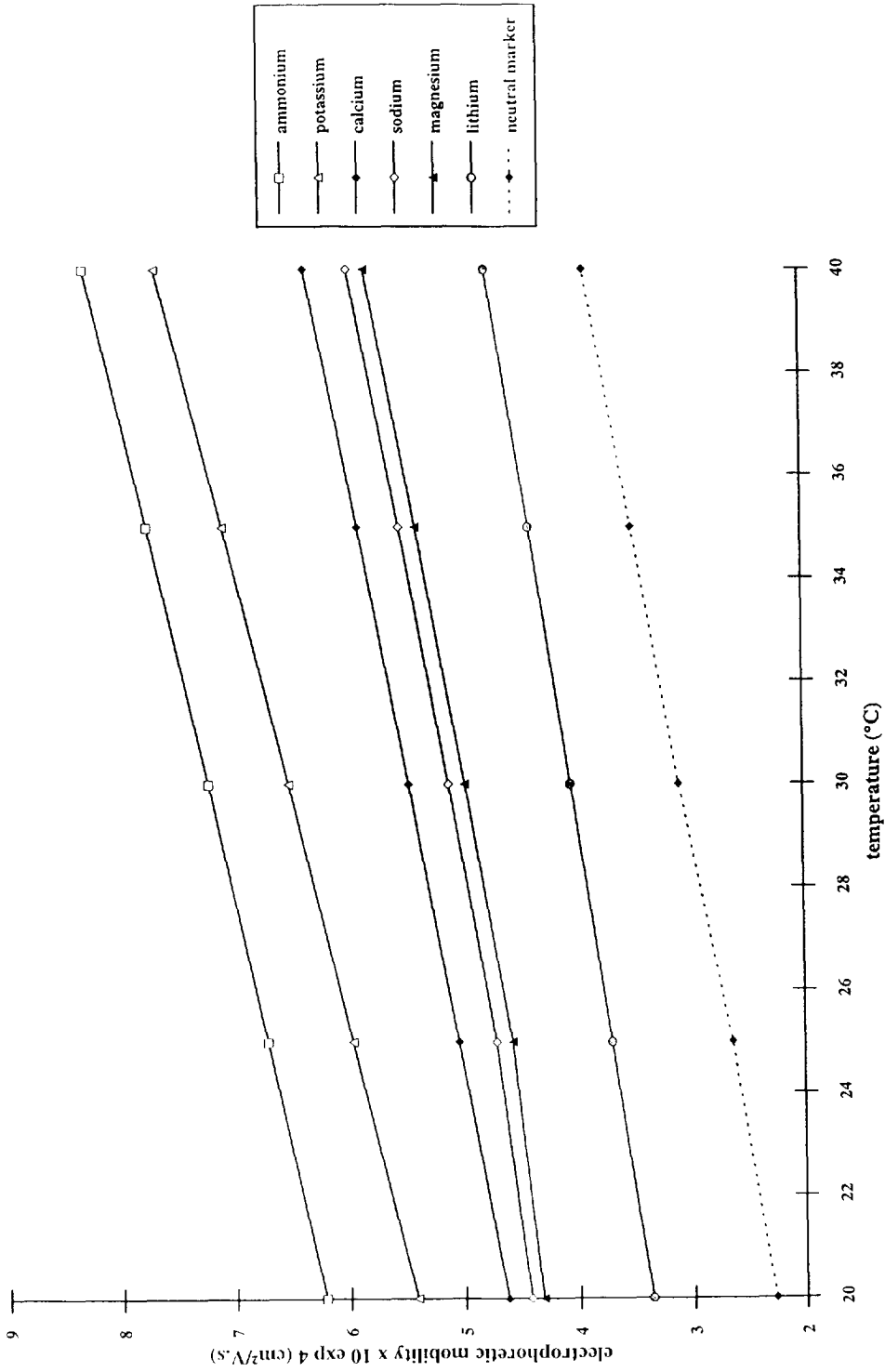


Fig. 7. Variation of electrophoretic mobility with temperature modification. Experimental conditions as in Fig. 6 except applied voltage (30 kV).

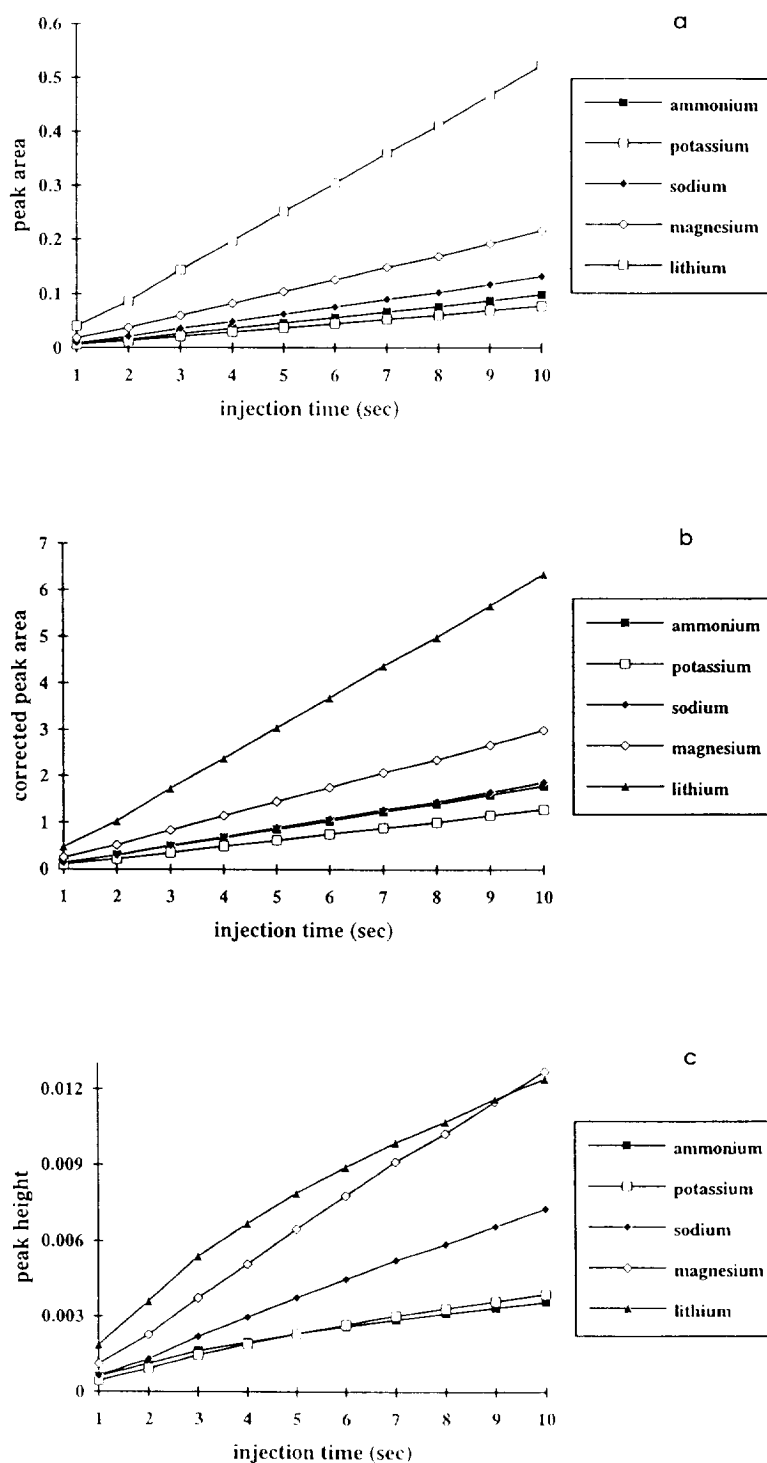


Fig. 8. Influence of injection time versus peak area, corrected peak area and peak height. Experimental conditions as in Fig. 6 except cation concentration (2 ppm). (a) Peak area; (b) corrected peak area; (c) peak height.

Table 5  
Influence of hydrodynamic injection time on peak height, peak area and corrected peak area of several cations

Cation	Slope	Intercept	Correlation coefficient
<i>Corrected peak area</i>			
Ammonium	0.183	-0.054	0.99990
Potassium	0.132	-0.036	0.99987
Sodium	0.192	-0.066	0.99981
Magnesium	0.304	-0.069	0.99991
Lithium	0.654	-0.228	0.99989
<i>Peak area</i>			
Ammonium	0.010	$-3.5 \times 10^{-3}$	0.99964
Potassium	0.008	$-2.7 \times 10^{-3}$	0.99974
Sodium	0.013	$-4.9 \times 10^{-3}$	0.99974
Magnesium	0.022	$-5.3 \times 10^{-3}$	0.99984
Lithium	0.054	-0.018	0.99988
<i>Peak height</i>			
Ammonium	0.00032	$5.6 \times 10^{-4}$	0.98852
Potassium	0.00038	$2.6 \times 10^{-4}$	0.99374
Sodium	0.00074	$-5.3 \times 10^{-4}$	0.99932
Magnesium	0.00013	$-1.6 \times 10^{-4}$	0.99955
Lithium	0.00011	$1.6 \times 10^{-4}$	0.98935

Experimental conditions as in Fig. 8.

Table 6  
Slopes and correlation coefficients of calibration plot for quantitative analysis of several cations

Cation	Slope	Intercept	Correlation coefficient
<i>Corrected peak area</i>			
Ammonium	0.140	-0.095	0.99960
Potassium	0.080	-0.041	0.99978
Calcium	0.143	-0.071	0.99952
Sodium	0.132	-0.041	0.99950
Magnesium	0.244	-0.224	0.99977
Lithium	0.502	-0.510	0.99975
<i>Peak area</i>			
Ammonium	$7.64 \times 10^{-3}$	$-6.18 \times 10^{-3}$	0.99966
Potassium	$4.77 \times 10^{-3}$	$-2.81 \times 10^{-3}$	0.99981
Calcium	$9.56 \times 10^{-3}$	$-4.46 \times 10^{-3}$	0.99956
Sodium	$9.42 \times 10^{-3}$	$-7.02 \times 10^{-3}$	0.99982
Magnesium	$17.51 \times 10^{-3}$	$-16.59 \times 10^{-3}$	0.99977
Lithium	$40.99 \times 10^{-3}$	$-53.75 \times 10^{-3}$	0.99920
<i>Peak height</i>			
Ammonium	$1.3 \times 10^{-4}$	$1.28 \times 10^{-3}$	0.97537
Potassium	$1.4 \times 10^{-4}$	$9.2 \times 10^{-4}$	0.97841
Calcium	$2.7 \times 10^{-4}$	$1.81 \times 10^{-3}$	0.97656
Sodium	$3.6 \times 10^{-4}$	$1.11 \times 10^{-3}$	0.99060
magnesium	$8.0 \times 10^{-4}$	$1.24 \times 10^{-3}$	0.99438
Lithium	$4.5 \times 10^{-4}$	$4.18 \times 10^{-3}$	0.97609

Experimental conditions as in Fig. 9.



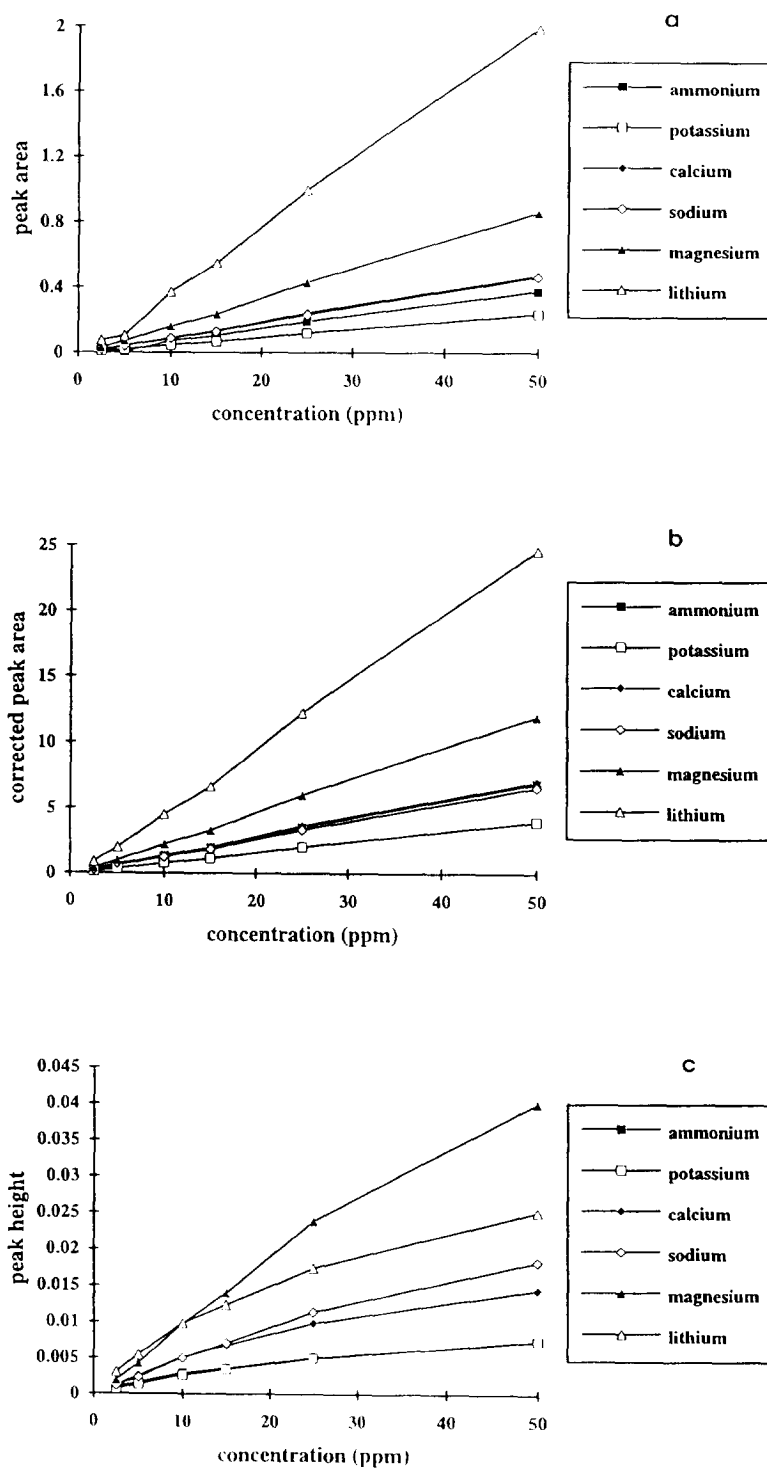


Fig. 9. Linear calibration plots for inorganic cations. Fused-silica capillary dimensions, 57 cm (50 cm to detector)  $\times$  75  $\mu$ m I.D., 100  $\mu$ m  $\times$  800  $\mu$ m aperture; electrolyte, 10 mM imidazole, 2.5 mM 18-crown-6, pH 4.5; indirect UV detection, 214 nm; applied voltage, 25 kV; hydrodynamic injection, 2 s. (a) Peak area; (b) Corrected peak area; (c) Peak height.

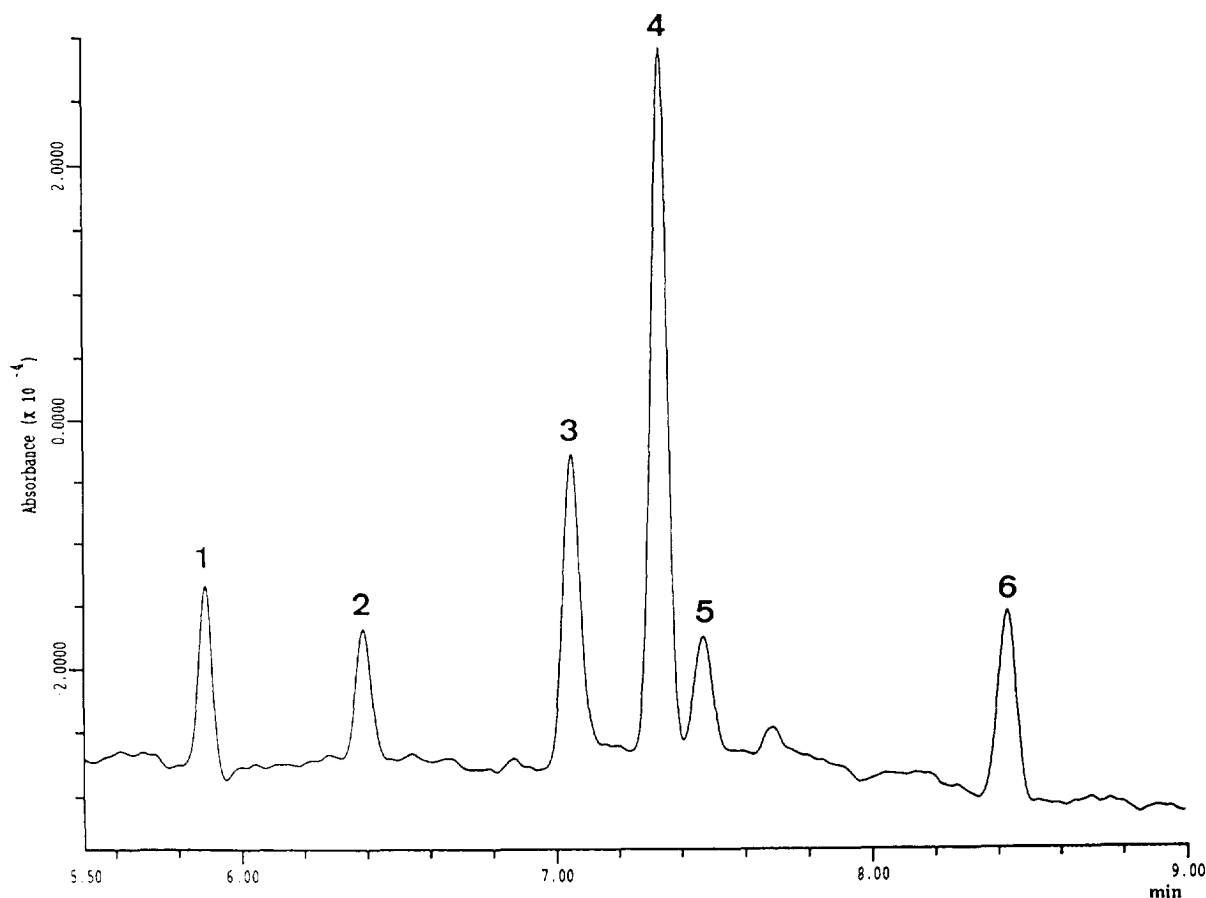


Fig. 10. Separation of an inorganic cation mixture at 10-ppb level. Experimental conditions as in Fig. 9 except hydrodynamic injection (2 s). Cations: 1 =  $\text{NH}_4^+$ ; 2 =  $\text{K}^+$ ; 3 =  $\text{Ca}^{2+}$ ; 4 =  $\text{Na}^+$ ; 5 =  $\text{Mg}^{2+}$ ; 6 =  $\text{Li}^+$ .

coefficient always reaches 0.999 for all cations when corrected peak area and peak area were used (Table 6). As previously stated, for the cations that have an electrophoretic mobility close to that of imidazole (sodium and magnesium), their peak height versus concentration is a linear function up to 25 ppm. The correlation coefficients are equal to 0.999 in the 2.5–25 ppm concentration range. Beyond this value, we observed a loss of linearity due to the limited column capacity. Lastly, an important decrease in efficiency was also observed when the solute concentration was increased from 2.5 to 50 ppm. For example, a loss in peak efficiency of 94% for ammonium and 93% for lithium occurred. This

loss in peak efficiency was less important for sodium and magnesium (54% and 64%, respectively).

Although the peak efficiency decreases with increasing injection time, the separation of an inorganic cation standard is possible at low concentrations (10 ppb) without loss of resolution, by increasing the hydrodynamic injection time up to 20 s (Fig. 10).

#### 4. Conclusion

The separation of ammonium, alkali and alkaline-earth cations by CE is easily achieved

with an imidazole-based electrolyte to which 18-crown-6 has been added. The separation of potassium–ammonium or strontium–barium is favoured by complex formation with 18-crown-6. The determination of the stability complex constant with 18-crown-6 for several cations can be experimentally determined by CE from electrophoretic mobilities versus  $-\log[18\text{-crown-6}]$  curves. The high stability constants for potassium, barium and strontium induce in a lower migration velocity than those of ammonium and other cations. So, during the analysis of complex matrix samples, the crown ether concentration appears to be a very convenient parameter to monitor the selectivity of cations.

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