

Separation of transition metal cations by capillary electrophoresis

Optimization of complexing agent concentrations (lactic acid and 18-crown-6)

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Abstract

The simultaneous separation of alkali, alkaline earth and transition metal cations by capillary electrophoresis was achieved using an electrolyte containing imidazole as indirect UV co-ion and lactic acid and 18-crown-6 as complexing agents with indirect UV detection. The addition of lactic acid to the imidazole electrolyte allows the separation of transition metal cations, whereas 18-crown-6 allows one to resolve ammonium and potassium and also sodium and lead. An electrolyte containing 10 mM imidazole, 5 mM lactic acid and 0.5 mM 18-crown-6 at pH 4.5 and with the separation temperature fixed at 30°C allow the simultaneous separation of alkali, alkaline earth and transition metal cations by capillary electrophoresis. By increasing the hydrodynamic injection time to 15 s, a linear relationship between corrected peak area and cation concentration in the 0.1–1 ppm range was obtained for transition metal cations. Further, the separation of inorganic cations at a low concentration level (100 ng ml⁻¹) was achieved without any loss of resolution.

1. Introduction

Capillary electrophoresis (CE) is very useful for the separation of inorganic cations [1,2]. The analytical approach is based on the indirect detection mode because of the transparency of inorganic cations in the UV region and on separation using operating conditions such that the electroosmotic flow must be in the same direction as the electrophoretic mobility of the

ions analysed in order to minimize their migration time. In order to use indirect photometric detection and to obtain symmetrical peaks, a UV-absorbing cationic compound having a similar electrophoretic mobility to that of the analyte cations must be selected as the main constituent of the electrolyte. Beck and Engelhardt [3] first proposed the imidazolium cation as a chromophore co-ion of the electrolyte for the separation of alkali and alkaline earth metal cations. We have previously studied the separation of ammonium, alkali and alkaline earth metal cations by capillary electrophoresis with an imidazole-

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based electrolyte to which 18-crown-6 was added [4]; Optimization of the 18-crown-6 concentration was achieved. The crown ether concentration appears to be a very convenient parameter to monitor the selectivity of cations (K^+/NH_4^+ , Sr^{2+}/Ba^{2+}) during the analysis of complex matrix aqueous samples. Adding 2.5 mM 18-crown-6 to the 10 mM imidazole system (pH 4.5) generally gives the maximum resolution of ammonium and potassium cations; nevertheless, it is possible to shift the potassium peak in the electropherogram by adding up to 300 mM of 18-crown-6. The complexation constants of several cations with 18-crown-6 can be experimentally determined by capillary electrophoresis from electrophoretic mobilities versus $-\log[18\text{-crown-6}]$ curves [4]. The high stability constants for potassium ($\log K_s = 2.1$), barium ($\log K_s = 3.6$) and strontium ($\log K_s = 2.7$) explain their lower migration compared with those of ammonium and other cations.

Transition metal cations and alkali and alkaline earth metal cations have similar electrophoretic mobilities. The separation of all these cations requires the addition of a complexing agent to the electrolyte, in order to decrease their electrophoretic mobility by in-situ complexation. Table 1 reports some publications related to the separation of inorganic cations by capillary electrophoresis [5–13].

Shi and Fritz [5] compared different chromophores and complexing agents for the separation of alkali, alkaline earth, transition metal and lanthanide cations. Among selected UV-absorbing co-ions, phenylethylamine, benzylamine, *p*-toluidine and 4-methylbenzylamine were found to be the most satisfactory. They used α -hydroxyisobutyric (HIBA), phthalic, malonic, tartaric, lactic and succinic acids as complexing agents; among these, HIBA and phthalic acid gave higher selectivities. HIBA was previously used for the separation of several mono- and divalent cations [6], but good separations were subsequently obtained with phthalic acid [5]. It was found that even better separations of metal cations could be obtained with tartrate or lactate as complexing agent; an excellent separation of

alkali, alkaline earth and transition metal ions was achieved in less than 6 min using 8 mM 4-methylbenzylamine–15 mM lactate–5% methanol (pH 4.25).

In another study, the same group [7] resolved a metal cation mixture by capillary electrophoresis with 4-methylbenzylamine electrolyte containing lactic acid, 18-crown-6 and methanol; protonated 4-methylbenzylamine was used as a co-ion for indirect UV detection of the sample cations and for pH adjustment.

In order to obtain the separation of metal cations having similar electrophoretic mobilities, the equilibrium kinetics between the free metal cation and the complexed species must be very rapid to maintain a narrow analyte electrophoretic zone. If this equilibrium is too slow, the various chemical species of an analyte would migrate at different velocities and the electrophoretic zone of the analyte would become too broad. In a lactic acid-based electrolyte, no peak could be obtained for Al(III) cation owing to the slow kinetics of forming and dissociating the Al(III)–lactate complex. Thus, the non-complexing nicotinamide-based electrolyte allows the simultaneous separation of alkali, alkaline earth and Al(III) cations [7].

Lin et al. [8] studied the role of complexing agents (acetic, glycolic, lactic, α -hydroxyisobutyric, oxalic, malonic, tartaric and succinic acids) added to an imidazole-based electrolyte in the separation of alkali and alkaline earth metal cations. The best separations were achieved using malonic or succinic acid, but glycolic, lactic or HIBA acid also provide good separations. When the concentration of one analyte in the sample is particularly high (for instance, Na^+ matrix in a serum sample), the addition of oxalic or citric acid to the electrolyte will avoid the overlap of the large Na^+ peak with the minor peaks. In another study, Lee and Lin [9] used several background carriers (imidazole, pyridine, benzylamine) under acidic pH conditions during the separation of some metal cations and they selected glycolic acid rather than succinic acid as the complexing agent.

Recently, Yang et al. [10] reported the

Table 1
Selected examples of inorganic cation separations by capillary electrophoresis

Cations	Detection	Electrolyte	Separation time (min)	Ref.			
K ⁺ , Ba ²⁺ , Ca ²⁺ , Na ⁺ , Mg ²⁺ , Li ⁺ , Pb ²⁺ , Ni ²⁺ , Cd ²⁺ , lanthanides	Indirect UV, 214 nm	2 mM Phthalic acid–UV Cat 1–20% MeOH (pH 3.3)	12	[5]			
		2.5 mM Tartaric acid–6 mM <i>p</i> -toluidine–20% MeOH (pH 4.8)	9				
		8 mM 4-Methylbenzylamine–15 mM lactic acid–5% MeOH (pH 4.25)	6				
Alkali, alkaline earth, lanthanides, transition metals	Indirect UV, 214 nm	10 mM Waters UV Cat-1–4 mM HIBA (pH 4.4)	2	[6]			
Metal cations and ammonium	Indirect UV, 214 or 254 nm	7.5 mM 4-Methylbenzylamine–11 mM lactic acid–2.6 mM 18-crown-6 8% MeOH (pH 4.3)	7	[7]			
		8 mM Nicotinamide–0.95 mM 18-crown-6–12% MeOH (pH 3.2)	14				
K ⁺ , Ba ²⁺ , Ca ²⁺ , Na ⁺ , Mg ²⁺ , Li ⁺	Indirect UV, 215 nm	5 mM Imidazole–2.1 mM acetic acid (pH 6)	2	[8]			
		5 mM Imidazole–4.55 mM glycolic acid (pH 4)	2				
		5 mM Imidazole–5 mM lactic acid (pH 4)	2				
		5 mM Imidazole–6.4 mM HIBA (pH 4)	4				
		5 mM Imidazole–1.9 mM oxalic acid (pH 4)	2				
		5 mM Imidazole–3 mM succinic acid (pH 4.5)	2				
		5 mM Imidazole–4 mM malonic acid (pH 4)	2				
		5 mM Imidazole–3.6 mM malic acid (pH 3.7)	2				
		5 mM Imidazole–1.9 mM tartaric acid (pH 4)	2				
		Li ⁺ , Na ⁺ , K ⁺ , Cs ⁺ , Mg ²⁺ , Sr ²⁺ , Ca ²⁺ , Ba ²⁺ , Mn ²⁺ , Fe ²⁺ , Co ²⁺ , Ni ²⁺ , Cu ²⁺ , Zn ²⁺ , Cd ²⁺ , Ag ⁺ , Al ³⁺ , Pb ²⁺	Indirect UV, 210 nm		10 mM Imidazole–13 mM glycolic acid (pH 4)	15	[9]
		254 nm	10 mM Pyridine–12 mM glycolic acid (pH 4)		18		
210 nm	10 mM Benzylamine–16 mM glycolic acid (pH 4)	15					
210 nm	5 mM Imidazole–6.75 mM HIBA (pH 4)	12					
NH ₄ ⁺ , K ⁺ , Na ⁺ , Li ⁺ , Mg ²⁺ , Ca ²⁺ , Sr ²⁺ , Mn ²⁺ , Ni ²⁺ , Zn ²⁺ , Cu ²⁺	Indirect UV	5 mM Imidazole–0.53 mM–18-crown-6–6.5 mM HIBA–20% MeOH (pH 4.5)	10	[10]			
Alkali, alkaline earth, transition metal, lanthanides	Indirect UV, 214 nm	6 mM N,N-Dimethylbenzylamine–4.2 mM HIBA (pH 5)	11	[11]			
K ⁺ , Ba ²⁺ , Sr ²⁺ , Ca ²⁺ , Na ⁺ , Mg ²⁺ , Li ⁺ , NH ₄ ⁺ , Cs ⁺	Indirect UV, 254 nm	5 mM Benzimidazole–40 mM 18-crown-6–tartrate (pH 5.2)	4	[12]			
K ⁺ , Ba ²⁺ , Sr ²⁺ , Ca ²⁺ , Na ⁺ , Mg ²⁺ , Li ⁺ , NH ₄ ⁺	Indirect UV, 215 nm	4 mM Copper(II) sulfate–4 mM formic acid–4 mM 18-crown-6 (pH 3)	5	[13]			

simultaneous separation of ammonium and alkali, alkaline earth and transition metal cations in a background electrolyte system composed of imidazole, HIBA, 18-crown-6 and methanol. Finally, other workers employed 18-crown-6 or HIBA as complexing agents added to an electrolyte containing N,N-dimethylbenzylamine [11], benzimidazole [12] or copper(II) sulfate [13] as chromophore agent for the separation of inorganic cations.

In this work, we studied the influence of several physico-chemical parameters (imidazole, lactic acid and 18-crown-6 concentrations) on the separation of ammonium, alkali, alkaline earth and transition metal cations by capillary electrophoresis.

2. Experimental

2.1. Apparatus

Separations were carried out on a P/ACE 2210 apparatus (Beckman, Fullerton, CA, USA) equipped with a UV detector with wavelength filters (190, 200, 214, 254, 260 and 280 nm). Fused-silica capillaries (Beckman) of dimensions 75 μm I.D., 375 μm O.D. and 57 cm long (50 cm to the detector) were used. The part where the separation takes place was kept at a constant temperature by immersion in a cooling liquid circulating in the cartridge with a detection aperture of $100 \times 800 \mu\text{m}$. The solutes were injected at the anode end of the capillary in the hydrodynamic mode by nitrogen superpressure $3.45 \cdot 10^7 \text{ Pa}$ (0.5 p.s.i.). Indirect UV detection at 214 nm was performed by a light beam focused directly on the capillary with the detection window set 7 cm from the end of the capillary. An IWT computer and System Gold software version 7.11 (Beckman) were used for instrument control and for data collecting and processing. 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 Beckman pH meter (Model ϕ 10).

2.2. Chemicals

Imidazole (99% purity) was obtained from Sigma (St. Louis, MO, USA) and 18-crown-6 (99%) and lactic acid (85%) from Aldrich (Milwaukee, WI, USA). The water used in the preparation of electrolytes and that necessary for dilutions was of HPLC quality (Fisons, Milan, Italy). The electrophoretic electrolyte pH was adjusted to the desired pH by adding a 1 M acetic acid stock solution (Carlo Erba, Milan, Italy). Each buffer and rinsing solution was filtered before use through a membrane filter having a diameter of 25 mm and a porosity of 0.2 μm (Whatman, Maidstone, UK).

3. Results and discussion

The electrolyte used for the separation of inorganic cations (alkali, alkaline earth and transition metal) must contain an organic cation which has an electrophoretic mobility close to that of the analytes in order to obtain symmetrical and efficient electrophoretic peaks, and also which possesses an intense chromophore group in the UV region. Imidazole is a nitrogen heterocyclic compound ($\text{p}K_{\text{a}1} = 6.9$ and $\text{p}K_{\text{a}2} = 14.5$) whose UV absorption spectrum has a maximum set at 211 nm ($\epsilon = 3300 \text{ l mol}^{-1} \text{ cm}^{-1}$ at pH 4.5). The electrophoretic mobility of the imidazolium cation ($45.8 \cdot 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$) was determined in 10 mM sodium acetate electrolyte at pH 4.5; this UV-absorbing co-ion has an electrolyte mobility close to those of Mg^{2+} and Na^+ (46.3 and $48.10 \cdot 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, respectively), which were measured in 10 mM imidazole electrolyte (pH 4.5) [4].

3.1. Effect of imidazole concentration

In recent paper [4], a study of the influence of the imidazole electrolyte concentration at pH 4.5 on selectivity in the range 1–15 mM was reported. The values of the resolution between $\text{K}^+/\text{Ca}^{2+}$, $\text{Ca}^{2+}/\text{Na}^+$, $\text{Na}^+/\text{Mg}^{2+}$ and $\text{Mg}^{2+}/\text{Li}^+$ are reported in Table 2. In the imidazole con-

Table 2
Influence of imidazole concentration on resolution of alkali and alkaline earth metal cation mixture

Imidazole concentration (mM)	Resolution (R_s)			
	K^+/Ca^{2+}	Ca^{2+}/Na^+	Na^+/Mg^{2+}	Mg^{2+}/Li^+
1	6.42	5.34	0.66	5.02
2	6.53	3.62	0.15	6.55
5	14.34	6	1.07	10.92
7	15.65	5.71	1.91	10.64
10	21.95	5.47	3.38	13.21
12	23.76	5.2	4.16	13.98
15	28.02	4.55	5.44	14.92

Fused-silica capillary dimensions, 57 cm (50 cm to detector) \times 75 μ m I.D., 100 μ m \times 800 μ m aperture; electrolyte, imidazole (pH 4.5); indirect UV detection, 214 nm; applied voltage, 15 kV; temperature, 25°C; hydrodynamic injection, 2 s; cation concentration, 5 ppm.

centration range 1–5 mM, the resolution between sodium and magnesium cations is always too low ($0.66 < R_s < 1.07$). An increase in imidazole concentration from 7 to 12 mM improves the separation between strontium and calcium cations and also between nickel and zinc cations (Fig. 1), but the resolution between these two last transition metal cations always remains < 1 . Some transition metal cations [manganese, iron(II), cobalt, lead] co-migrated under such non-complexing conditions. Consequently, the addition of a complexing agent (lactic acid) to the electrolyte (pH 4.5) is necessary to resolve these cations.

3.2. Effect of lactic acid concentration

The concentration of lactic acid added to the electrolyte (10 mM imidazole, pH 4.5) would change the degree of complexation of the transition metal cations. By increasing the lactic acid concentration from 1 to 12 mM in the running electrolyte, we observed a decrease in the electrophoretic mobilities of some metal cations [nickel, zinc, cobalt, iron(II), copper]; for example, a 33% electrophoretic mobility loss was observed for copper cation. Under such conditions, the electrophoretic mobilities of other

cations (cadmium, manganese) remain constant (Fig. 2).

An increase in lactic acid concentration would change the migration order of cobalt, nickel, zinc, cadmium and lithium cations. Thus, nickel and zinc migrated between cadmium and cobalt cations in the lactic acid concentration range 1–3 mM, then migrated between cadmium and lithium for lactic acid concentrations < 7 mM and finally migrated after lithium for lactic acid concentrations > 7 mM. According to the nature of the sample matrix, we can shift the nickel and zinc peaks relative to the other cation peaks by adjusting the lactic acid concentration.

In our work, the best resolution was obtained by selecting 5 mM rather than 10 mM as the lactic acid concentration (Fig. 3).

Finally, if the addition of lactic acid to the imidazole electrolyte improves the separation of transition metal cations, another complexing agent (18-crown-6) must be added to this electrolyte to resolve not only ammonium and potassium cations, but also sodium and lead cations.

3.3. Effect of 18-crown-6 concentration

As is well known, the separation of K^+/NH_4^+ or Sr^{2+}/Ba^{2+} is favoured by complex formation

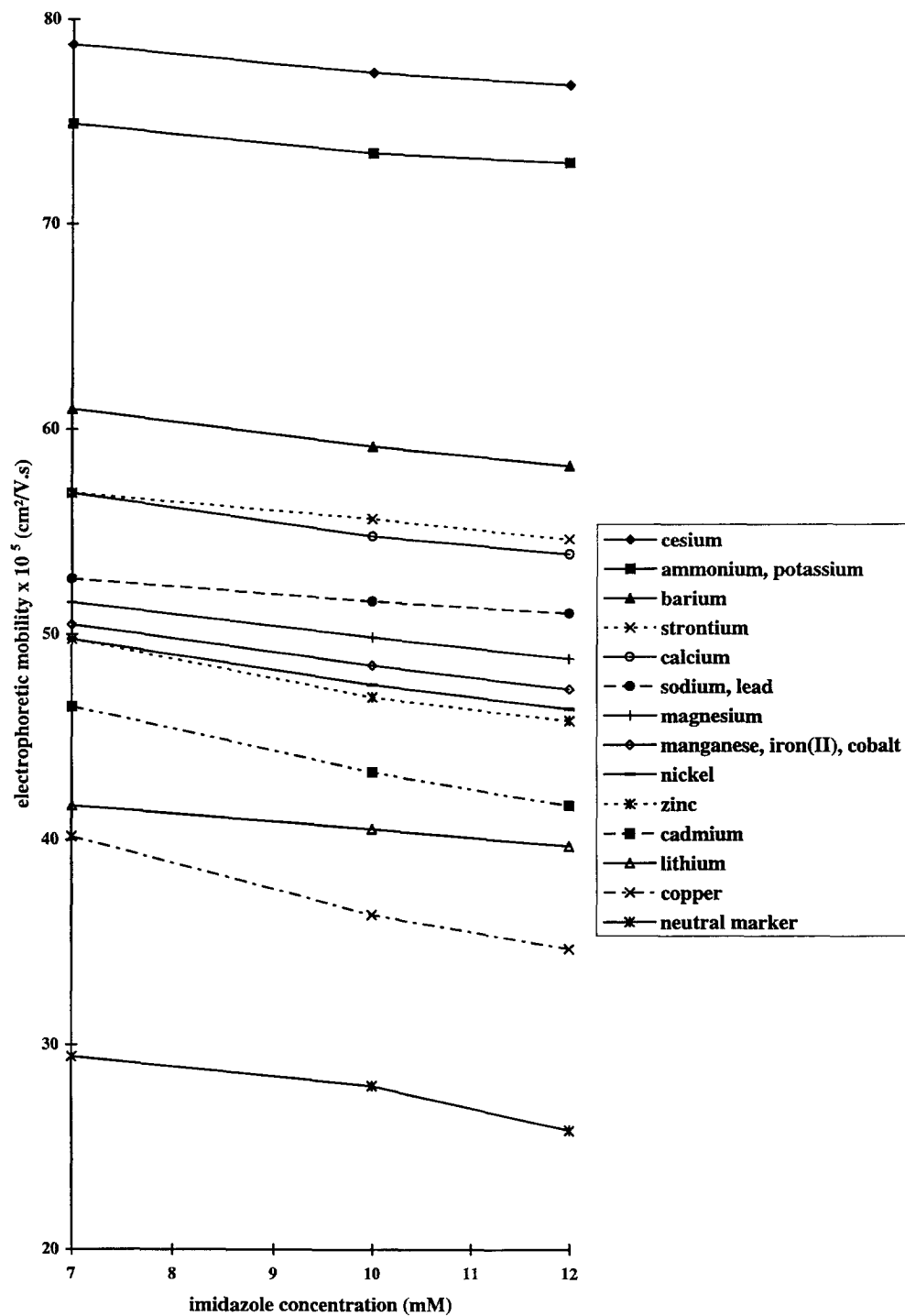


Fig. 1. Influence of imidazole concentration on the electrophoretic mobility of 17 inorganic cations. Fused-silica capillary dimensions, 57 cm (50 cm to detector) \times 75 μ m I.D., 100 μ m \times 800 μ m aperture; electrolyte, imidazole (pH 4.5); indirect UV detection, 214 nm; applied voltage, 20 kV; temperature, 30°C; hydrodynamic injection, 2 s; cation concentration, 5 ppm except for caesium and lead (10 ppm) and barium and copper (20 ppm).

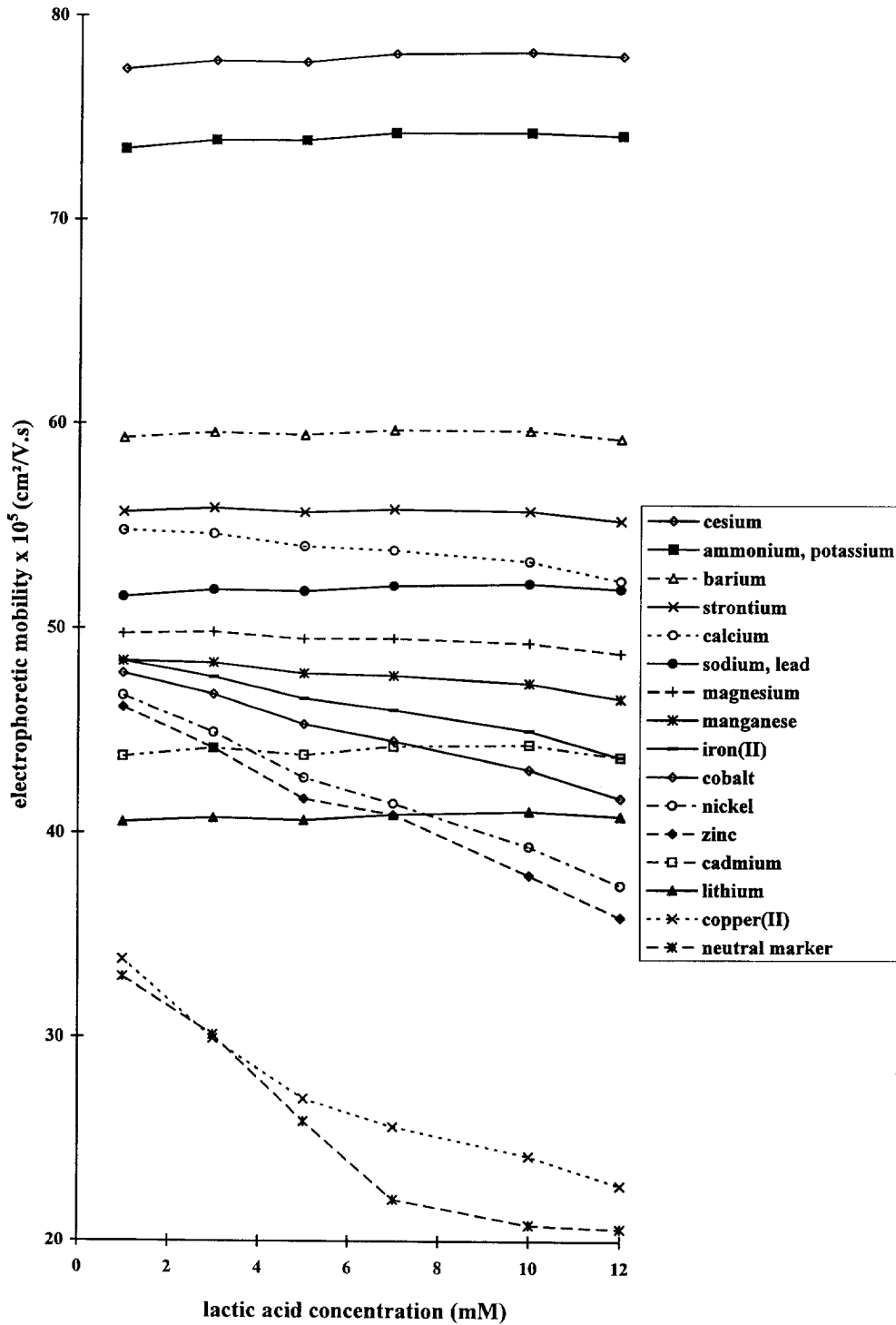


Fig. 2. Influence of lactic acid concentration on the electrophoretic mobility of 17 inorganic cations. Experimental conditions as in Fig. 1 except electrolyte composition, 10 mM imidazole–lactic acid (pH 4.5).

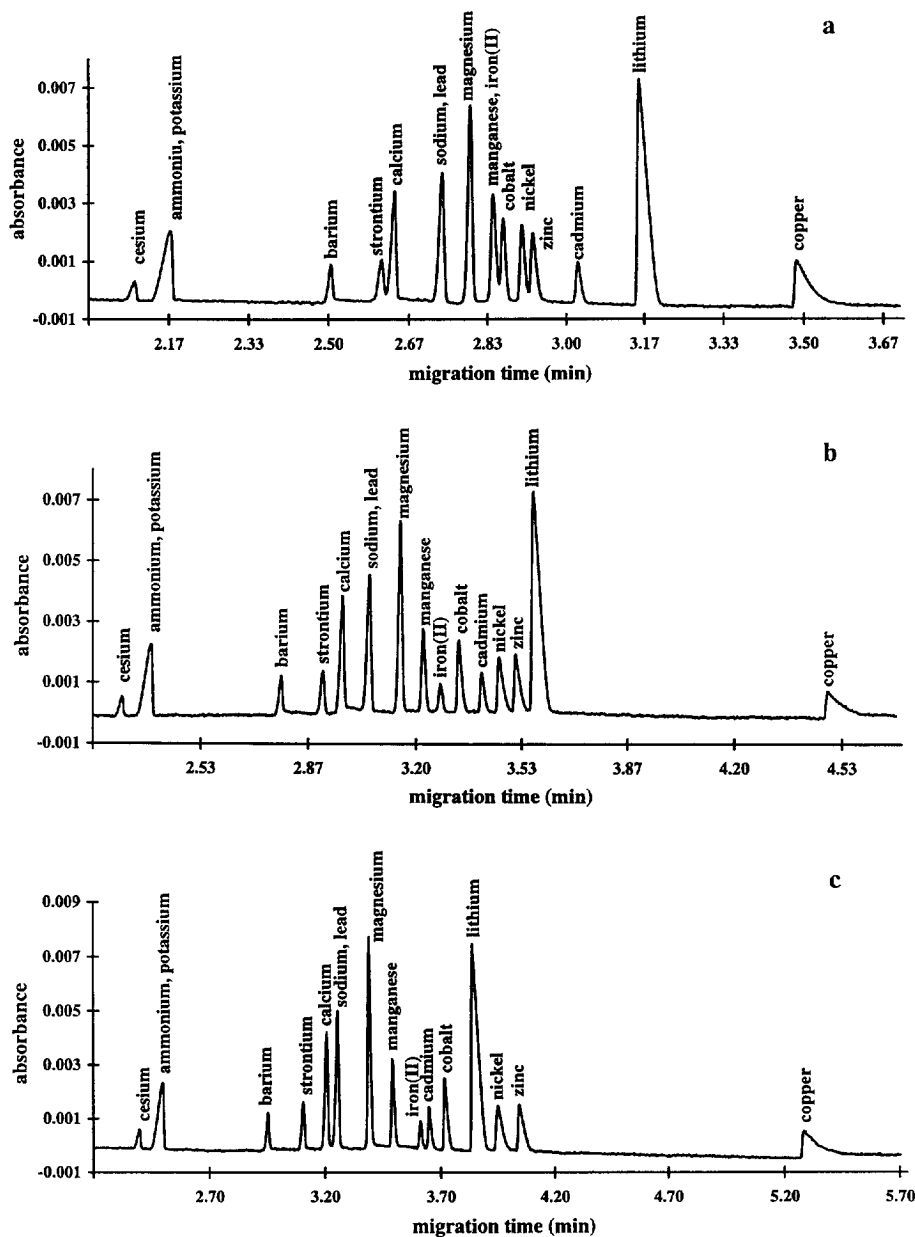


Fig. 3. Influence of lactic acid concentration on the separation of iron(II), cobalt and cadmium cations. Experimental conditions as in Fig. 2, except lactic acid concentration: (a) 1 mM; (b) 5 mM; (c) 10 mM.

with 18-crown-6 [4]. We studied the influence of the 18-crown-6 concentration in the range 0.5–1.5 mM on the selectivity by keeping the same imidazole and lactic acid concentrations (10 and 5 mM, respectively, at pH 4.5).

The addition of 18-crown-6 to the imidazole-

based electrolyte containing lactic acid affects the migration of almost all cations except K^+ and NH_4^+ . Thus, a low concentration of 18-crown-6 (0.5 mM) added to the imidazole electrolyte induces a migration time increase of 8%, 24% and 47% for Sr^{2+} , Ba^{2+} and Pb^{2+} , respectively

(Figs. 3b and 4a) due to the complexation of these cations with 18-crown-6.

Finally, increasing the 18-crown-6 concentra-

tion from 0.5 to 1.5 mM induces a modification of migration order (Fig. 4); thus, strontium cation migrates faster than sodium cation at 0.5

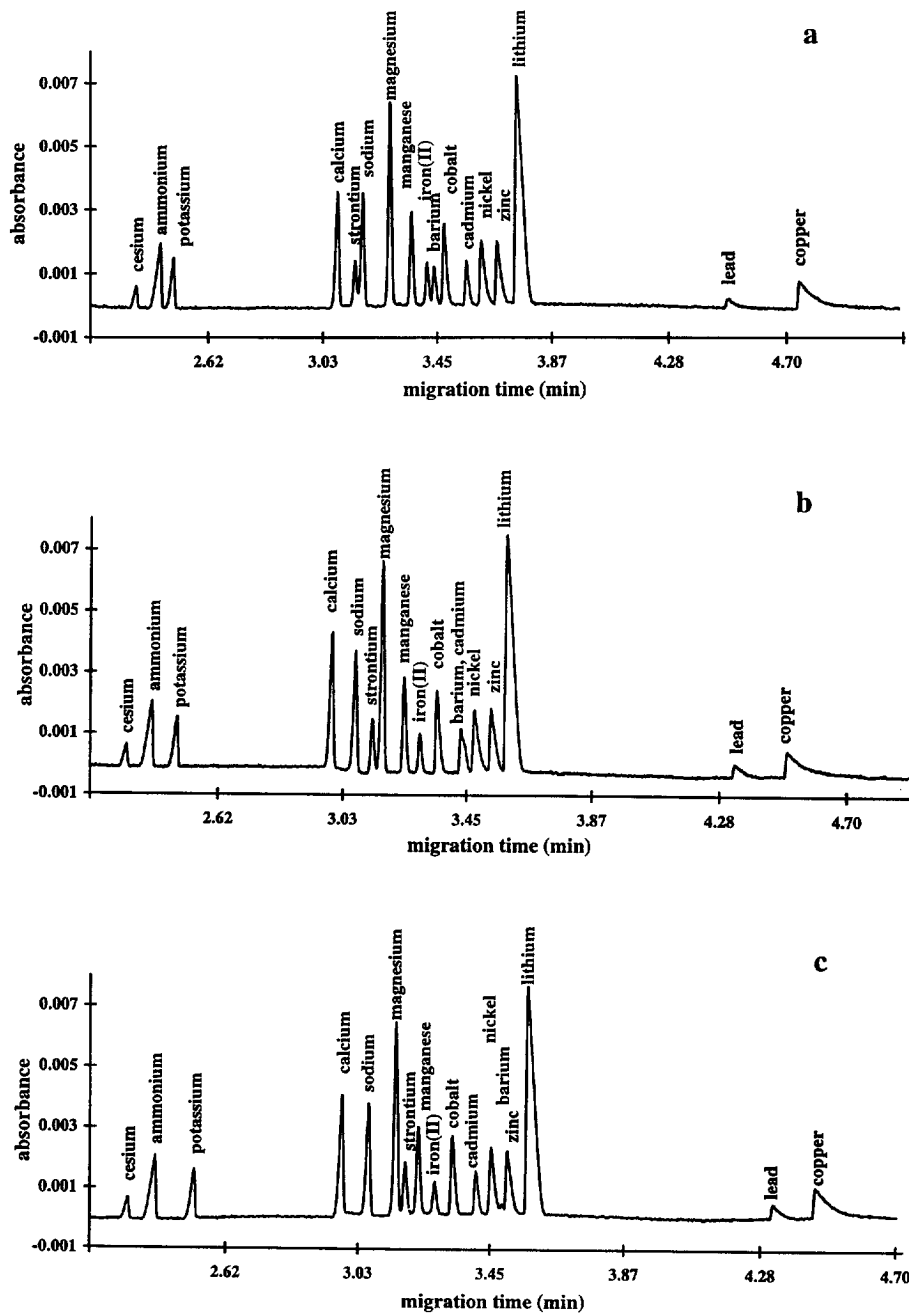


Fig. 4. Effect of 18-crown-6 concentration on the separation of strontium, barium and lead cations. Experimental conditions as in Fig. 3 except electrolyte, 10 mM imidazole–5 mM lactic acid (pH 4.5), and 18-crown-6 concentration: (a) 0.5 mM; (b) 1 mM; (c) 1.5 mM.

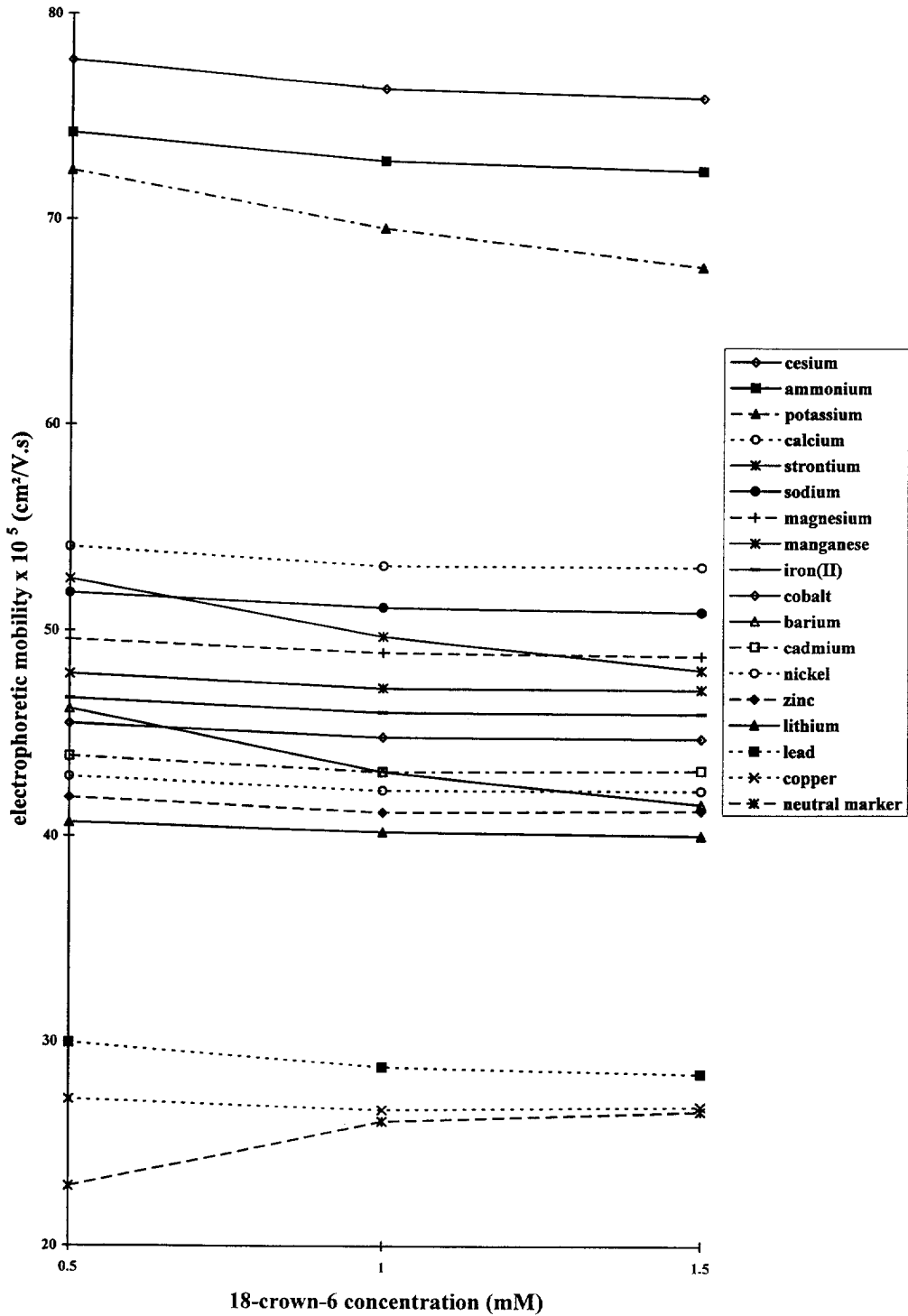


Fig. 5. Influence of 18-crown-6 concentration on the electrophoretic mobility of 17 inorganic cations. Experimental conditions as in Fig. 4.

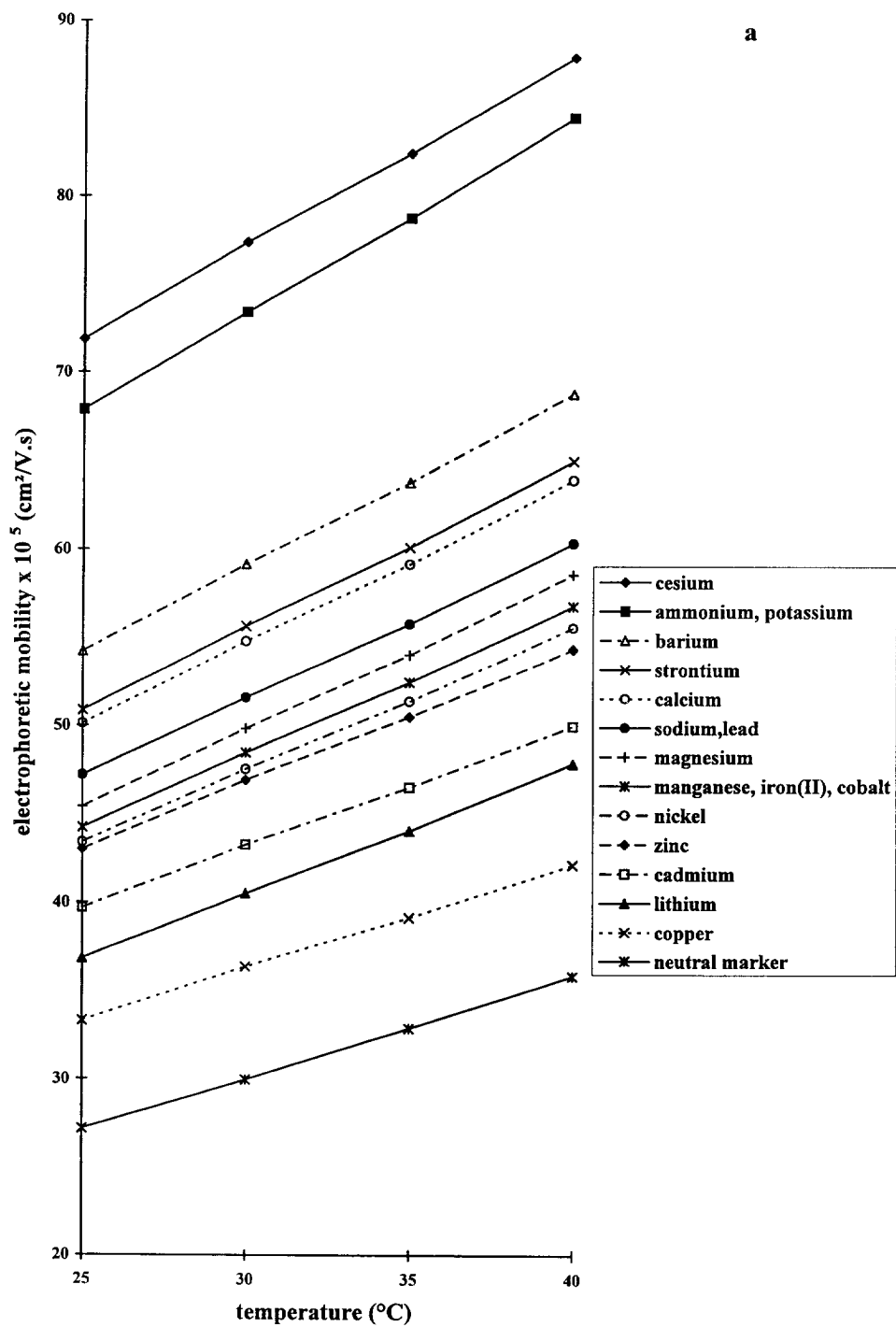


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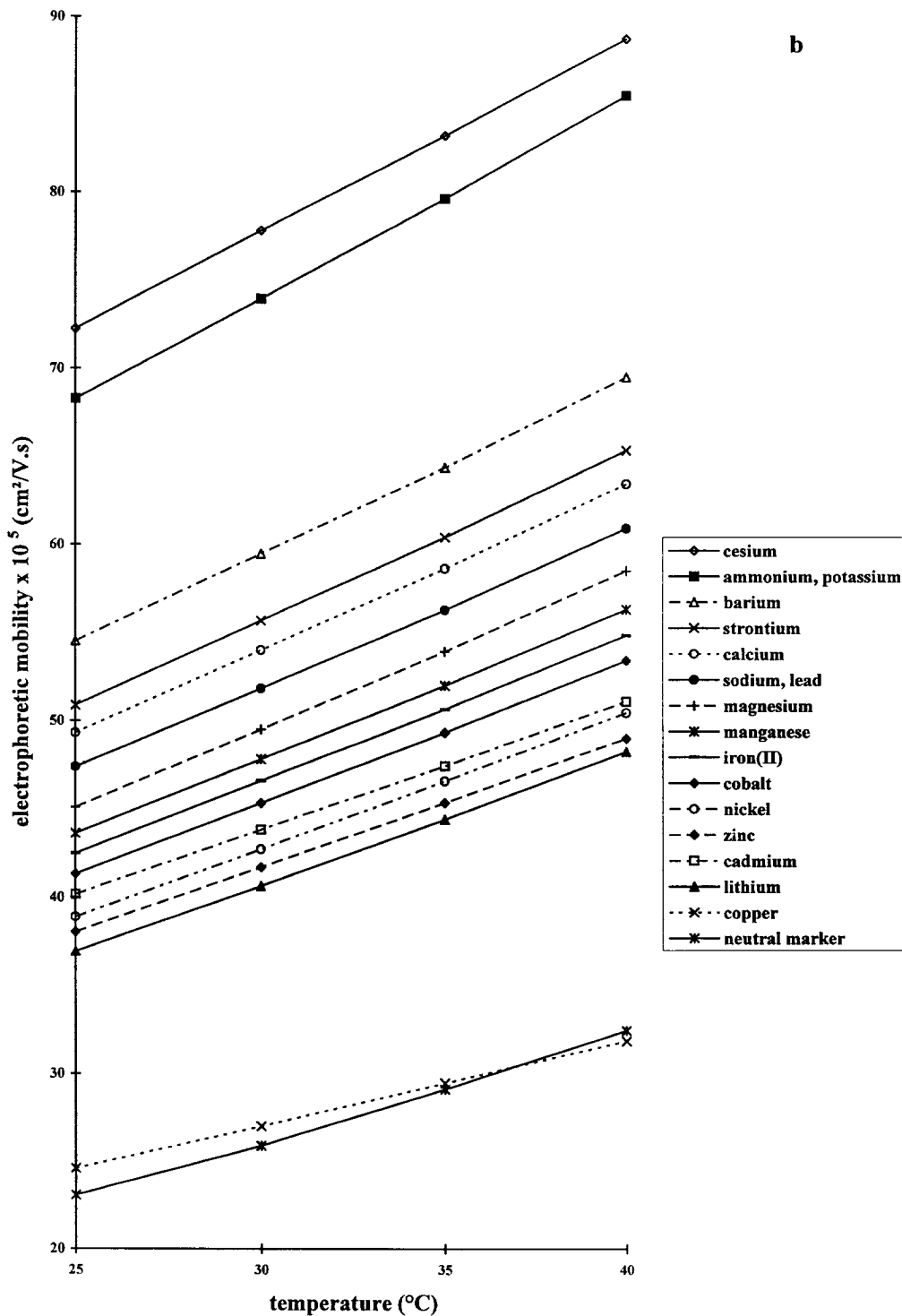


Fig. 6.

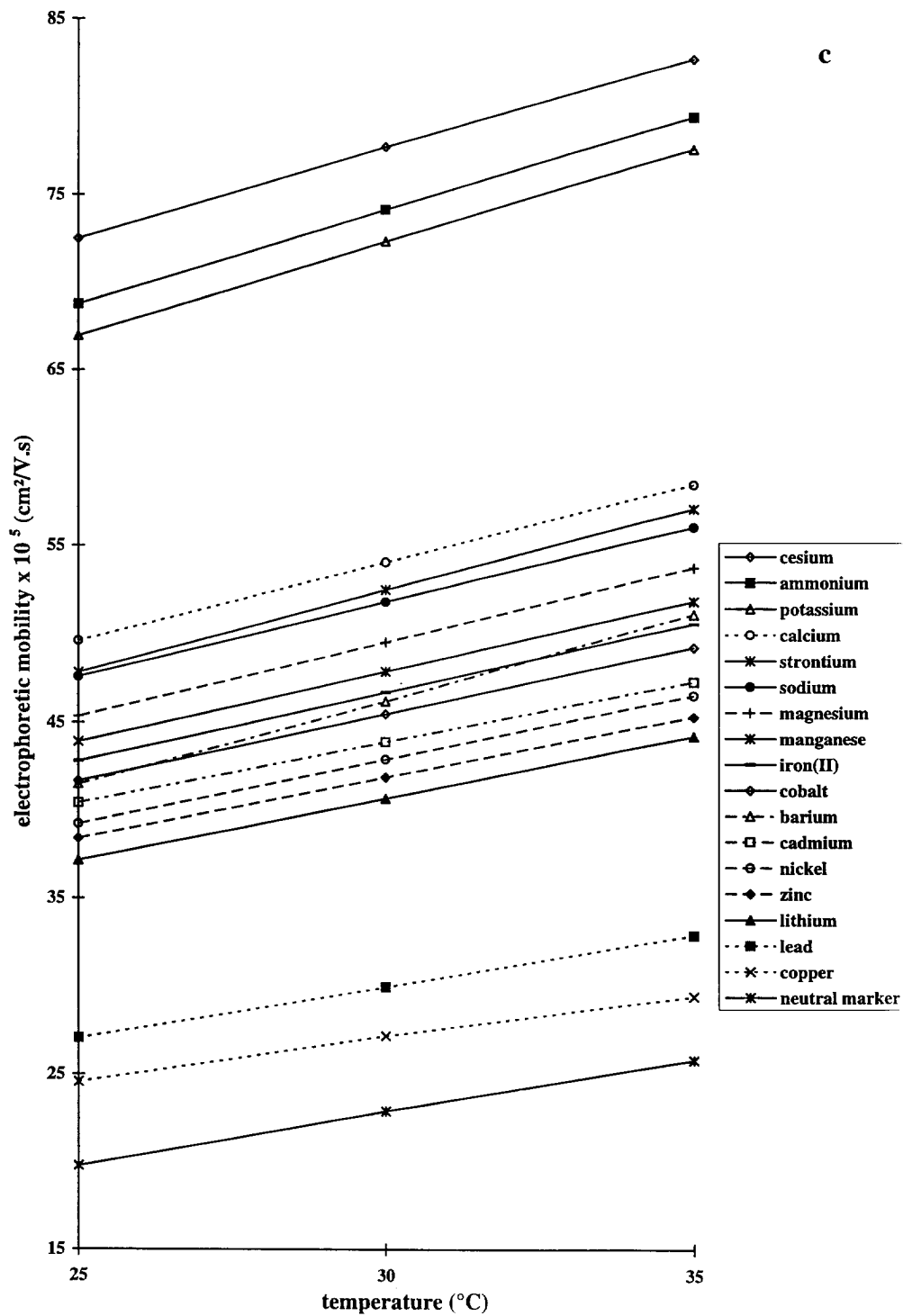


Fig. 6. Dependence of cation electrophoretic mobility on separation temperature. Fused-silica capillary dimensions, 57 cm (50 cm to detector) \times 75 μm I.D., 100 μm \times 800 μm aperture; indirect UV detection, 214 nm; applied voltage, 20 kV; hydrodynamic injection, 2 s; cation concentration, 5 ppm except for caesium and lead (10 ppm) and barium and copper (20 ppm). (a) 10 mM imidazole (pH 4.5); (b) 10 mM imidazole–5 mM lactic acid (pH 4.5); (c) 10 mM imidazole–5 mM lactic acid–0.5 mM 18-crown-6 (pH 4.5).

mM 18-crown-6 and faster than manganese cation at 1.5 mM 18-crown-6. Also, the electrophoretic mobility of the barium cation decreases when the 18-crown-6 concentration increases, but co-migrates with cadmium at 1 mM 18-crown-6 (Fig. 5). The 18-crown-6 concentration variation affects only the electrophoretic mobility of these two cations.

3.4. Effect of temperature

The effect of temperature on the separation was studied for three different electrolytes (10 mM imidazole, 5 mM lactic acid–10 mM imidazole and 0.5 mM 18-crown-6–5 mM lactic acid–10 mM imidazole). Temperature variations affect physical parameters such as electrolyte viscosity, analyte diffusion coefficient, and consequently,

the electroosmotic flow and electrophoretic mobility of the analytes.

Fig. 6 shows the variation of electrophoretic mobility versus separation temperature in the range 25–40°C. For the different electrolytes, when the temperature increases from 25 to 40°C, the cation migration times decrease and so their electrophoretic mobilities increase.

The influence of separation temperature on resolution depends on the electrolyte composition. With the 10 mM imidazole electrolyte, a significant resolution improvement (142%) between nickel and zinc peaks was observed when the temperature was increased from 25 to 40°C. In contrast, no temperature variation improves the separation between manganese, iron(II) and cobalt cations. Using the electrolyte containing 10 mM imidazole and 5 mM lactic acid, no

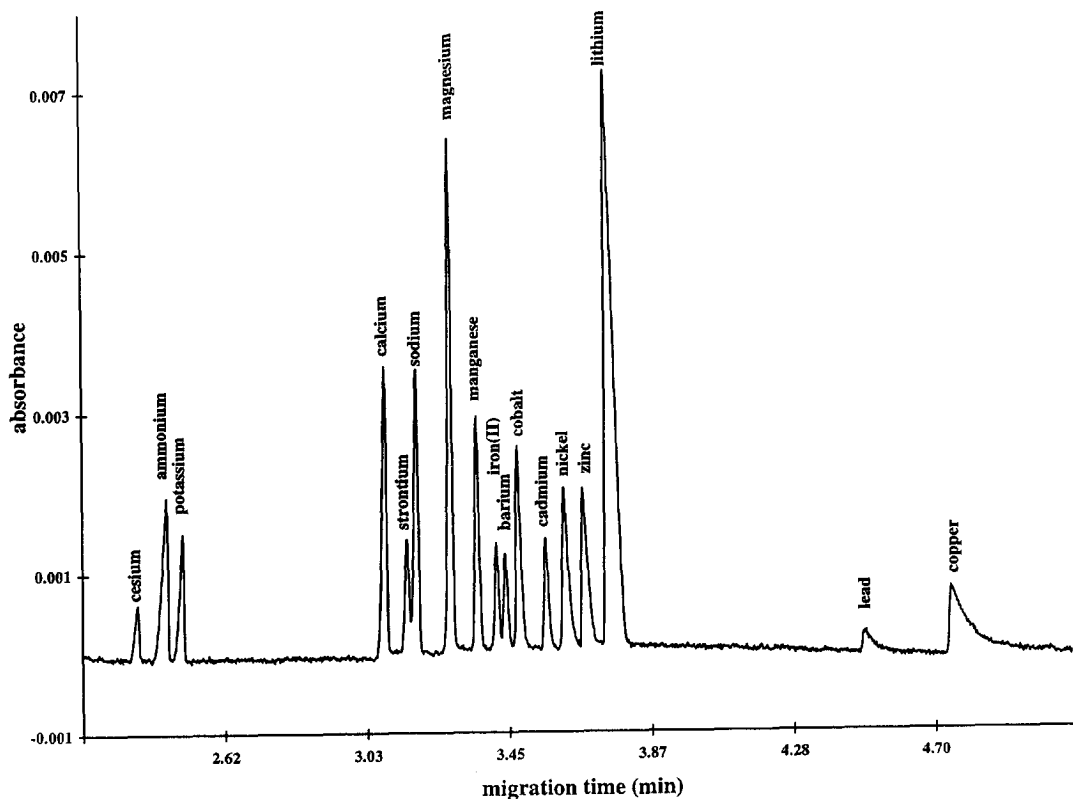


Fig. 7. Separation of a 17 inorganic cation mixture under optimized conditions. Experimental conditions as in Fig. 6c except temperature, 30°C.

significant modification of the resolution was observed with increase in temperature. Finally, using the electrolyte containing imidazole and the two complexing agents (lactic acid and 18-crown-6), the temperature is an important parameter for optimizing the separation of those

cations. Thus, the resolution between strontium and sodium cations increases with increase in temperature from 25°C ($R_s = 0$) to 30°C ($R_s = 1.23$). Moreover, we can shift the barium peak relative to the other peak by increasing the temperature; for instance, barium cation mi-

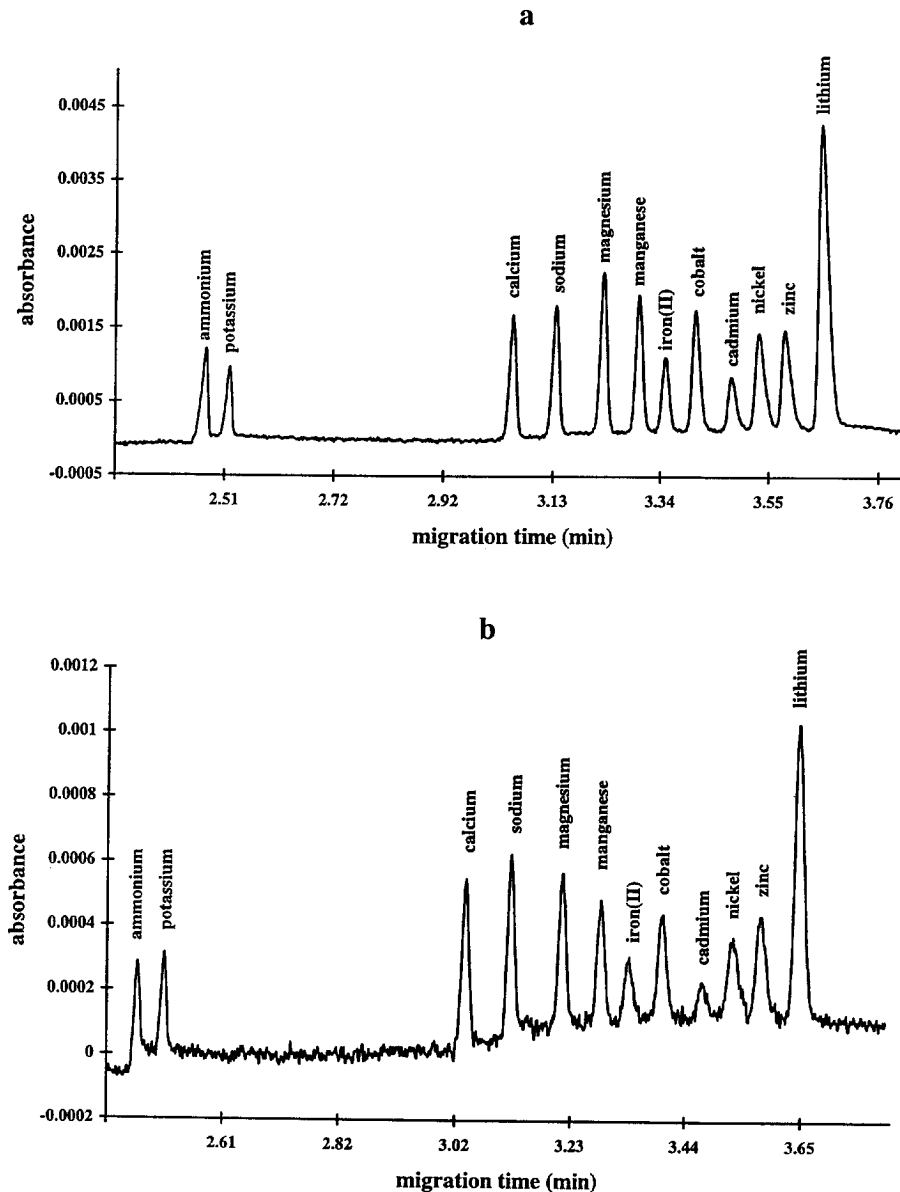


Fig. 8. Separation of standard mixture of 11 inorganic cations at sub-ppm concentrations by capillary electrophoresis. Experimental conditions as in Fig. 7 except cation concentrations: (a) ammonium, alkali and alkaline earth 0.5 ppm and transition metal 1 ppm; (b) ammonium, alkali and alkaline earth 50 ppb and transition metal 100 ppb.

Table 3
Parameters of linear calibration plots for several transition metal cations in the 0.1–1 ppm range

Cation	Slope (1/ppm)	Intercept	Correlation coefficient
Manganese	1.097	0.0038	0.99984
Iron(II)	1.947	0.016	0.99964
Cobalt	1.165	0.02	0.99970
Cadmium	2.237	0.0681	0.99752
Nickel	1.206	0.014	0.99925
Zinc	1.252	0.0101	0.99847

Experimental conditions as in Fig. 7.

grates slower than cobalt cation at 25°C whereas this cation migrates faster than cobalt and iron(II) cations at 35°C. Consequently, we selected 30°C as the optimum separation temperature owing to a better resolution between barium and iron(II) cations at 30°C than 35°C (resolution 1.10, 1.01, respectively). Fig. 7 shows the separation of alkali, alkaline earth and transition metal cations under such optimized conditions.

3.5. Quantitative aspects

Calibration graphs were determined for several transition metal cations [manganese, iron(II), cobalt, cadmium, nickel and zinc] using a hydrodynamic injection time of 15 s. These experiments were carried out using a 10 mM imidazole-based electrolyte containing the two complexing agents 5 mM lactic acid and 0.5 mM 18-crown-6. For all cations, the correlation coefficients were greater than 0.997 (Table 3). With this injection time (15 s), the separation of a sub-ppm inorganic cation mixture may be achieved without any resolution loss (Fig. 8).

4. Conclusion

The separation of ammonium, alkali, alkaline earth and transition metal cations was achieved by capillary electrophoresis with an imidazole-based electrolyte, to which two different complexing agents were added. Selectivity modifica-

tion was obtained by varying the concentration of each complexing agent, particularly during the analysis of complex matrix samples.

Whereas the separation of alkali and alkaline earth metal cations was successfully achieved with an imidazole electrolyte (10 mM, pH 4.5), the addition of 5 mM lactic acid to this imidazole electrolyte is required to resolve manganese, iron(II), cobalt, cadmium, nickel and zinc cations. Further, the addition of 0.5 mM 18-crown-6 to this imidazole–lactic acid electrolyte is necessary to resolve sodium and lead cations and also ammonium and potassium cations. Finally, the separation temperature appears also to be a very convenient parameter for monitoring the selectivity of these cations.

Acknowledgements

The authors thank the Eurothermes Society (La Bourboule, France) for support of this work and Mrs. D. Depernet for technical assistance.

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