

Separation of alkali and alkaline earth metal and ammonium cations by capillary zone electrophoresis with indirect UV absorbance detection

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Abstract

Carrier electrolytes combining complexing effects of negatively charged counter ions and electroneutral 18-crown-6 were investigated in the separations of alkali and alkaline earth metal and ammonium cations by capillary zone electrophoresis with indirect UV absorption detection. The use of tartrate with 18-crown-6 at a 0.04 mol l^{-1} concentration permitted the complete resolution of these cations in one electrophoretic run. Benzimidazole served as a visualization co-ion for indirect detection at 254 nm. The separation of some alkali and alkaline earth metal and ammonium cations in rain, tap and mineral water samples illustrates the application potential of the proposed approach.

1. Introduction

Separation methods are widely used in the analysis of alkali and alkaline earth metal and ammonium cations in various types of samples. At present, ion chromatography has a dominant position among these methods. However, capillary electrophoretic techniques have been shown to be promising alternatives for the analysis of this group of cations and capillary isotachopheresis (ITP) [1–10] and capillary zone electrophoresis (CZE) [11–18] seem particularly useful.

The ionic mobilities of alkali and alkaline earth metal and ammonium cations in aqueous electrolyte solutions under non-complexing conditions are close [19–21]. Therefore, some of them (K^+ , Rb^+ , Cs^+ and NH_4^+ , Ca^{2+} and Sr^{2+} , Mg^{2+} and Na^+) are difficult to separate when

the ionic mobilities determine their effective mobilities. Although methanol provides favourable differences in the ionic mobilities of the cations of interest [6,7,21], mostly aqueous electrolyte systems introducing complex equilibria are preferred in their electrophoretic separations. Here, negatively charged ligands [1,2,4,8,10,11–13,17,18] or electroneutral crown ethers [3,9,15] have been proposed for separations in the cationic mode. In ITP, some of the alkaline earth metal cations were separated in the anionic mode by converting them into anionically migrating chelates [22].

Only ITP is known to provide a complete electrophoretic resolution of alkali and alkaline earth metal and ammonium cations in one run [10]. Here, the separation is assumed to be due to the formation of weak complexes of the cations with polyethylene glycol. Similar interactions are involved in the electrophoretic sepa-

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rations of these cations when crown ethers are present in the electrolyte systems. However, the results obtained, *e.g.*, for 18-crown-6 in ITP [23], indicate that the differentiating power of this agent differs slightly from that of polyethylene glycol and the formation of mixed zones for some of the cations can be expected. For example, from these data it is predictable that at a concentration of 18-crown-6 in the carrier electrolyte as used by Baechmann *et al.* [15] a complete resolution of Cs^+ , K^+ , Rb^+ and NH_4^+ is problematic.

This work was aimed at investigating CZE separations of alkali and alkaline earth metal and ammonium cations in which complexing effects of negatively charged ligand(s) and electroneutral crown ethers were combined. It was expected that such a combination of the complexing agents would provide separation conditions suitable for the resolution of the complete group of the cations. Indirect UV absorption detection was chosen to monitor the separations.

2. Experimental

2.1. Instrumentation

A CS Isotachophoretic Analyzer (Labeco-Villa, Spišská Nová Ves, Slovak Republic) was used in the single-column configuration of the separation unit. The column was provided with a 300 μm I.D. capillary tube made of fluorinated ethylene-propylene copolymer (FEP). Its length from the injection point to the detector was 250 mm. The column was connected to a hydrodynamically closed counter-electrode compartment as is current in ITP equipment [21]. The samples were injected with the aid of laboratory-made devices for electromigration injection. A UV spectrophotometric detector [24] was used to monitor the separations at 254 nm.

2.2. Chemicals

The chemicals used for the preparation of the carrier electrolytes were obtained from BDH (Poole, UK), Serva (Heidelberg, Germany),

Janssen Chimica (Beerse, Belgium), Sigma (St. Louis, MO, USA), Lachema (Brno, Czech Republic), Reachim (Moscow, Russian Federation), Carlo Erba (Milan, Italy) and Spolana (Neratovice, Czech Republic). Demineralized methylhydroxyethylcellulose (Laboratory of Environmental Analysis, Comenius University, Bratislava, Slovak Republic) was used as an anticonvective additive to the carrier electrolyte. The solutions of the electrolytes were prepared from demineralized water.

Benzimidazole (Lachema), used for the preparations of the carrier electrolytes, was purified by precipitation from a methanolic solution. Other chemicals were used in purities as received.

2.3. Samples

A rain water sample was kindly provided by the Slovak Meteorological Institute (Bratislava, Slovak Republic). Tap water samples were collected in the laboratory in polyethylene sample containers. Mineral waters were purchased in a local supermarket. The samples were injected for analysis without any pretreatment.

3. Results and discussion

3.1. Separation conditions

For already mentioned reasons, the electrolyte systems studied in the CZE separations of alkali and alkaline earth metal and ammonium cations combined the complexing effects of negatively charged counter ions and uncharged 18-crown-6. To achieve a complete resolution of these cations, the choice of the counter-ion constituent(s) and the concentrations of the complexing agents was based on the following considerations.

(i) ITP measurements by Tazaki *et al.* [23] imply that a higher concentration of 18-crown-6 in the carrier electrolyte is favourable as it widens the range of the effective mobilities of the cations and at the same time provides a complete resolution of K^+ , Rb^+ , Cs^+ and NH_4^+ . From these measurements it is also apparent that the effective mobilities of alkaline earth metal

cations should be decreased via an appropriately chosen (complexing) counter-ion constituent to avoid the risk of the formation of mixed zones of these separands with the monovalent cations.

(ii) Although the concentration of crown ether in the carrier electrolyte is not restricted by the electrophoretic separation, the use of indirect detection sets the upper concentration limit for the counter-ion constituent. This is clear from the equation [25] relating the concentration limit of detection (c_{LOD}),

$$c_{\text{LOD}} = c_{\text{V}} / (DR \cdot TR) \quad (1)$$

with the concentration of the visualization co-ion (c_{V}), dynamic reserve (DR) and transfer (displacement) ratio (TR). From this equation it is apparent that favourable detection conditions require a low concentration of the co-ion in the carrier electrolyte and, consequently (electroneutrality), also a low concentration of the (complexing) counter ions.

(iii) The concentration of the complex forming counter ion in the carrier electrolyte has a direct influence on the effective mobilities of the separated cations (see, *e.g.*, refs. 26 and 27).

Therefore, weak complexing agents, although providing CZE resolutions of alkaline earth metal cations (see, *e.g.*, refs. 11 and 12), are not convenient for the intended group retardation.

With the above facts in mind, the counter-ion constituents (Table 1) were chosen among the acids with pH buffering capacities at $\text{pH} \approx 5.0$ – 6.0 that form complexes with alkaline earth metal cations with stability constants in the range 10^2 – 10^4 [28].

The effective mobility of the co-ion in the carrier electrolyte should be as close as possible to those of the separated ions [29,30]. In addition, favourable detection conditions require that its concentration in this electrolyte is low (see Eq. 1) while its molar absorptivity at the detection wavelength is high [30,31]. Undoubtedly, these criteria restrict the choice and there is hardly any constituent that meets them for all of the separated cations. The co-ions employed in this work are given in Table 1.

Twenty electrolyte systems based on the constituents listed in Table 1 (each with and without 18-crown-6) were tested [32]. Although several of these systems provided complete resolutions of the cations, only two of them (Table 2) gave

Table 1
Co-ions and counter ions studied as carrier electrolyte constituents in the separation of cations

Ions	Species ^a	Rating	Remarks
Co-ions	Benzimidazole	+	+ = Suitable
	Benzylamine	–	– = Drifts on the baseline, noise of the signal
	Cytosine	–	
	Creatinine	–	
	2,4,6-Collidine	+	
	2-Aminopyridine	–	
Counter ions	ADA	–	+ = Suitable
	PIPES	–	– = Insufficient differentiation
	FMIDA	–	
	HIDA	–	
	Glycolic acid	–	
	Chloroacetic acid	–	
	Iminodiacetic acid	–	
	Tartaric acid	+	
	Citric acid	– ^b	

^a ADA = N-(2-acetamido)iminodiacetic acid; PIPES = 1,4-piperazinebis(ethanesulphonic) acid; FMIDA = phosphonomethyliminodiacetic acid; HIDA = hydroxyethyliminodiacetic acid.

^b Strong retardation of doubly charged cations.

Table 2
Electrolyte systems

Parameter	Carrier electrolyte ^a	
	No. 1	No. 2
Solvent	Water	Water
Co-ion	Benzimidazole	Collidine
Concentration (mmol l ⁻¹)	5	5
Counter ion	Tartrate	Tartrate
pH	5.2	5.2
Additive	HEC; <i>m</i> -HEC	HEC; <i>m</i> -HEC
Concentration (%, w/v)	0.1; 0.1	0.1; 0.1
Complexing additive	Crown ether	Crown ether
Concentration (mmol l ⁻¹)	40	40

^a Collidine = 2,4,6-collidine; HEC = hydroxyethylcellulose; *m*-HEC = methylhydroxyethylcellulose; crown ether = 18-crown-6.

an acceptable performance also from the point of view of detection. Strong (often reproducible) drifts of the baselines on the electropherograms and/or increased noise of the detector restricted our choice (see also below).

Ba²⁺, Ca²⁺, Sr²⁺ and Mg²⁺ ions migrate in aqueous electrolyte systems under non-complexing conditions or in the presence of weak complexing agents with effective mobilities intermediate between those of K⁺ and Na⁺ ions [10–12,19–21]. The use of tartrate in the carrier electrolytes changed this migration configuration by decreasing the effective mobilities of the alkaline earth metal cations relative to the other separands (Figs. 1a and 2a). This group retardation was favourable as complete resolutions could be achieved via complexing effects of 18-crown-6 (Figs. 1b and 2b).

From the electropherograms in Fig. 1, it can be seen that the migration times of the cations increased on addition of crown ether to the carrier electrolyte. This is in agreement with the ITP measurements [9,23], which showed that the crown-complexed cations have lower ionic mobilities than the solvated cations. The shorter migration times for Ca²⁺, Sr²⁺, Mg²⁺ and Li⁺ in electrolyte system 2 [relative to those obtained in

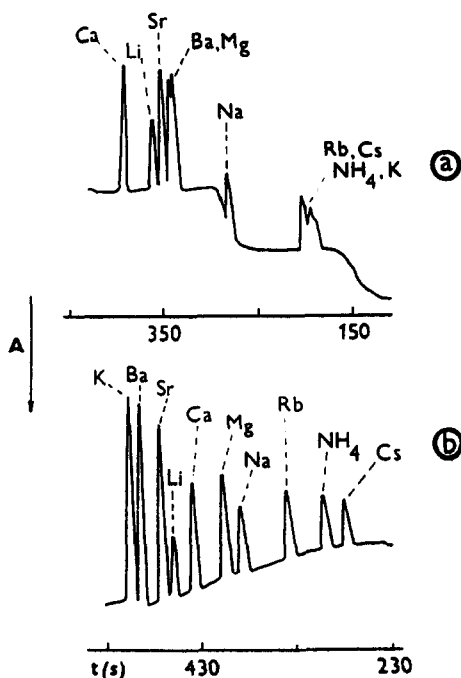


Fig. 1. Electropherograms from the separation of a model mixture (each at a concentration of 10⁻⁴ mol l⁻¹) in electrolyte system (Table 2): (a) without crown ether; (b) with crown ether (0.04 mol l⁻¹). Driving current, 75 μ A; electromigration injection at 50 μ A (time = 4 s); FEP capillary column (25 cm \times 0.3 mm I.D.). A = decreasing absorption at 254 nm.

the carrier electrolyte without crown ether (Fig. 2a)], however, indicate the opposite effect of the crown complexation. In spite of the fact that competitions of the ligands for the metal cations have to be considered, these results suggest that complexation of the co-ion (collidine) by the crown ether also plays a rôle.

3.2. Separation performance

Drifts of the baseline in CZE separations with indirect UV absorption detection are ascribed to thermal effects due to Joule heating [30,33]. Our experiments carried out in this context (different driving currents) agreed with these findings [32] (see also Fig. 3). On the other hand, the electropherograms in Fig. 1 imply that this need not be the only explanation as the addition of 18-crown-6 to the carrier electrolyte (under other-

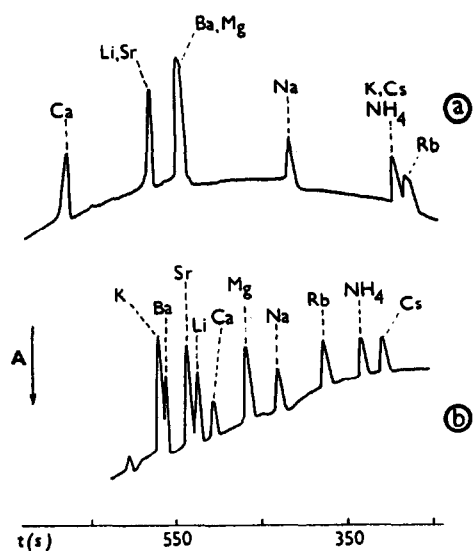


Fig. 2. Electropherograms from the separation of a model mixture (each at a concentration of 10^{-4} mol l^{-1}) in electrolyte system (Table 2): (a) without crown ether; (b) with crown ether (0.04 mol l^{-1}). Other conditions as in Fig. 1.

wise identical working conditions) improved the drift considerably (Fig. 1). However, for most of the tested systems the effect of the crown ether was not so significant. The electropherograms in Fig. 3 show that the resolutions of K^+ and Ba^{2+} and to a lesser extent Sr^{2+} and Li^+ depended on the driving current. Although decreased separation efficiencies at $10 \mu A$ (Table 3) provide a straightforward explanation for the decreased resolutions, different dependences of the effective mobilities of K^+ and Ba^{2+} on temperature should be also taken into the consideration for this particular pair.

The I.D. of the capillary tube employed in this work ($300 \mu m$) is less favourable from the point of view of thermal dispersion [34]. For less conductive carrier electrolytes, such as are currently used in separations with indirect detection (see Eq. 1), this need not be a serious disadvantage. The separation efficiencies obtained in our experiments (Table 3) and frontings of the peaks of the most mobile cations suggest that the electromigration dispersion (not related to the I.D. of the column [29]) was mainly responsible for the band broadening. These results also indicate that benzimidazole met the mobility

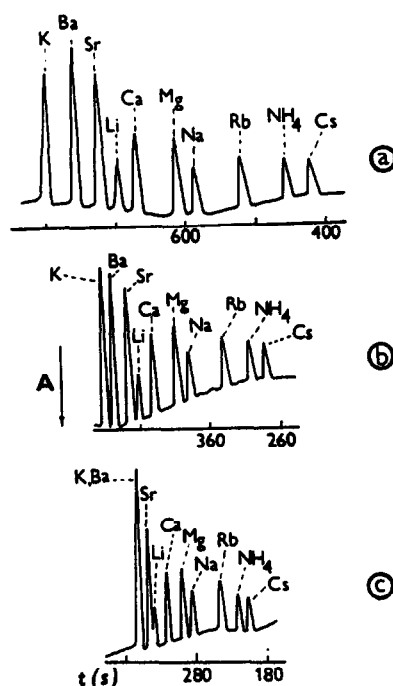


Fig. 3. Effect of the driving current on the CZE separations of the cations. The separations were carried out in electrolyte system 1 (Table 2). (a) $50 \mu A$; (b) $75 \mu A$; (c) $100 \mu A$. Electromigration injection at $40 \mu A$ (time = 4 s). Other conditions as in Fig. 1.

requirements only for less mobile separands. This is a general problem in CZE separations with indirect detection and probably the use of multi-component buffer systems [35] would eliminate this discriminative effect of the carrier electrolytes containing only one co-ionic constituent.

3.3. Application

Examples of CZE separations of practical samples under the proposed separating conditions are shown in Figs. 4 and 5. Although none of these samples contained all of the studied cations at detectable concentrations, the analytical potential of this approach is apparent. For example, it can be seen that the electrolyte systems combining complexation effects of various types of the ligands can provide enhanced selectivities of the separations and, thus, offer a

Table 3
Separation performance parameters obtained at different driving currents

Cation	50 μA		75 μA			100 μA	
	t_m (s)	N/m	t_m (s)	R.S.D. (%)	N/m	t_m (s)	N/m
Cs ⁺	421.0	46 400	288.0	0.16	43 500	207.5	35 300
NH ₄ ⁺	455.9	73 800	303.2	0.15	75 200	221.8	40 380
Rb ⁺	520.4	70 900	339.1	0.07	60 300	247.3	31 100
Na ⁺	581.6	84 600	387.3	0.13	122 700	286.6	67 300
Mg ²⁺	615.8	134 600	406.4	0.08	135 100	301.6	74 600
Ca ²⁺	669.7	159 200	438.5	0.05	157 200	322.5	66 200
Li ⁺	697.4	247 400	461.3	0.09	174 200	341.4	–
Str ²⁺	724.3	157 200	477.2	0.08	331 500	348.6	–
Ba ²⁺	757.9	292 200	498.0	0.10	361 300	364.3	–
K ⁺	800.0	325 600	511.1	0.07	380 400	364.3	–

t_m = Migration time; R.S.D. = relative standard deviation for five runs performed within one day; N/m = number of theoretical plates per metre.

way to develop very rapid procedures for the determination of alkali and alkaline earth metal and ammonium cations in various water samples. In this context, a detailed optimization of the composition of the carrier electrolyte is desirable.

Although the within-day reproducibilities of the migration times of the cations were very

favourable (Table 3) and their day-to-day fluctuations (1% or slightly less) could be ascribed to small changes in the composition of the carrier electrolyte, an improvement in the quantification is needed.

In spite of the fact that the injection time in

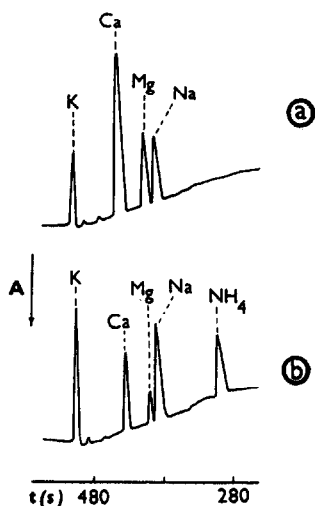


Fig. 4. Electropherograms from the separation of (a) drinking and (b) rain waters. Electrolyte system 1 (Table 2) was used. The separations were carried out with a driving current of 75 μA and electromigration injection was at 40 μA (time = 4 s).

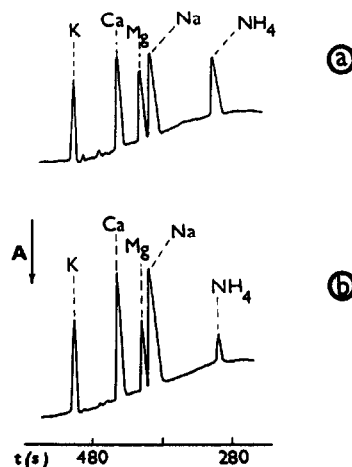


Fig. 5. Electropherograms from the separations of mineral waters in electrolyte system 1 (Table 2). (a) Baldovská mineral water (label concentrations: NH₄⁺ = 0.5 ppm, Na⁺ = 96.5 ppm, Mg²⁺ = 83.7 ppm, Ca²⁺ = 354.8 ppm, K⁺ = 20.0 ppm); (b) Santovská mineral water (label concentrations: NH₄⁺ = 0.2 ppm, Na⁺ = 372.4 ppm, Mg²⁺ = 82.7 ppm, Ca²⁺ = 440.9 ppm, K⁺ = 82.1 ppm). The driving current was 75 μA and electromigration injection was at 40 μA (time = 4 s).

the electromigration injection was carefully controlled (*ca.* 0.1% precision) and the injection current was stabilized to within 0.1% of the preselected value, the relative standard deviations for the peak areas for both model and practical samples were only seldom better than 7–10%. We consider that the main sources of these fluctuations were associated with the sample injection technique (extremely narrow bands of the separands focused at the end of the capillary which is moved from one solution to another). However, further studies along these lines are needed before the full potential of the proposed approach in quantitative analysis can be assessed.

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5. References

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