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New electrolyte systems for the determination of metal cations by capillary zone electrophoresis

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Abstract

Many +1 and +2 metal cations can be separated efficiently by CZE using lactic acid as a complexing reagent. Addition of a crown ether, as well as lactic acid, permits the separation of K^+ and NH_4^+ in addition to the ions previously separated. The problem of determining trace amounts of metal ions in the presence of a very high concentration of another metal ion was also addressed. For example, a large Na^+ peak (1000 ppm, w/w) covers up the peaks of Ca^{2+}, Mg^{2+}, Sr^{2+} and Ba^{2+} (1 ppm each). However, addition of increasing concentrations of methanol to the electrolyte permits complete resolution of Ca^{2+} and Mg^{2+} from the Na^+. Further addition of a crown ether moves Sr^{2+} and Ba^{2+} to longer migration times and permits resolution of these ions as well. Separation of metal cations with slower complexation kinetics is possible under conditions where only the free metal ions are present. Aluminum(III) and vanadium(IV), along with several other metal ions, were separated at pH 3.2 using nicotinamide as a buffer component and as a reagent for indirect detection.

1. Introduction

The art and science of separation of metal cations continues to develop at a fast pace. Indirect detection by means of a chromogenic cation [1,2] (often called a visualization reagent) is quite common, although suppressed conductivity [3] and electrochemical detection [4] have recently been used for some inorganic cations. It has become quite common to include a weak complexing reagent in the electrolyte to enhance the separation of cations with very similar electrophoretic mobilities. Several authors have reported excellent separations based on these principles [5–13]. In a recent publication we reported the separation of 27 metal cations in only 6.0 min using lactic acid as an auxiliary complexing reagent [13]. However, it was not possible to resolve the potassium and ammonium peaks from one another.

In the present work an electrolyte containing both lactic acid and a crown ether was used to separate 16 of the most commonly determined metal ions. The effect of methanol on this system was also investigated. Quantitative aspects of the separations were studied and the problem of determining trace metal ions in the presence of a high concentration of another cation was addressed. An uncomplexing electrolyte system is also introduced here.

2. Experimental

A Waters Quanta 4000 capillary electrophoresis system (Millipore Waters, Milford, MA, USA), equipped with a positive power supply was employed to separate metal cations and generate all electropherograms. Polyamide-

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coated, fused-silica capillaries (Polymicro Technology, Phoenix, AZ, USA), were 60 cm in length with an I.D. of 75 μm and a distance of 52.5 cm from the point of injection to the window of on column detection. Indirect UV detection was employed at 214 nm or 254 nm. A voltage of 15 to 30 kV was applied for separations. The time of hydrodynamic injection was 30 or 40 s for most separations. A Servogor 120 flatbed recorder (Goerz Instruments, Austria) was used to plot electropherograms. The chart speed was set at 3 cm/min for all electropherograms presented in this paper.

All standards and electrolytes were prepared with analytical-reagent grade chemicals and 18 MΩ deionized waters by a Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, USA).

Lactate buffers were mixed with lactic acid (J.T. Baker, Phillipsburg, NJ, USA), 4-methylbenzylamine (Fluka, Ronkonkoma, NY, USA), methanol (Fisher Scientific, Fair Lawn, NJ, USA) and 18-crown-6 (Aldrich, Milwaukee, WI, USA). The 4-methylbenzylamine was used as the protonated cation for indirect detection of the sample cations and for pH-adjustment. This reagent is identical to Waters UV-Cat 1 which is patented for use as an indirect detection reagent in capillary zone electrophoresis [14].

The uncomplexing buffer was prepared with nicotinamide (Sigma, St. Louis, MO, USA) and other modifiers. Formic acid (Aldrich, Milwaukee, WI, USA) was used to adjust pH.

3. Results and discussion

3.1. Electrolytes containing lactic acid and a crown ether

Lactic acid makes possible the separation of metal ions with almost identical mobilities by complexing the individual metal ions to varying degrees. The divalent transition metal ions and the lanthanides are two examples. However, NH_4^+ and K^+ cations also have virtually identical mobilities and are not complexed by lactic acid. Previous investigators found that ammonium and potassium ions can be separated by CZE if a suitable crown ether is incorporated into the electrolyte [15–18]. The K^+ ion is selectively complexed and its mobility is reduced just enough to permit a good separation.

We found that an electrolyte solution containing both lactic acid (11 m*M*) and a crown ether (2.6 m*M* 18-crown-6) will permit an excellent electrophoretic separation of 16 metal ions, including NH_4^+ and K^+ (Fig. 1). The electrolyte also contained 7.5 m*M* 4-methylbenzylamine as an indirect detection coion. Separations in which 12-crown-4 or 15-crown-5 was used in place of 18-crown-6 failed to separate NH_4^+ and K^+.

Incorporation of 18-crown-6 into the electrolyte containing lactic acid affects the migration of several metal ions other than K^+ and NH_4^+. The crown ether increases the migration time of Sr^{2+} by 15%, Pb^{2+} by 18% and Ba^{2+} by 35%, apparently by complexation to form a bulkier, less mobile species. The effect of 18-crown-6 on the migration times of metal ions, compared to lactic acid alone, is summarized in Table 1.

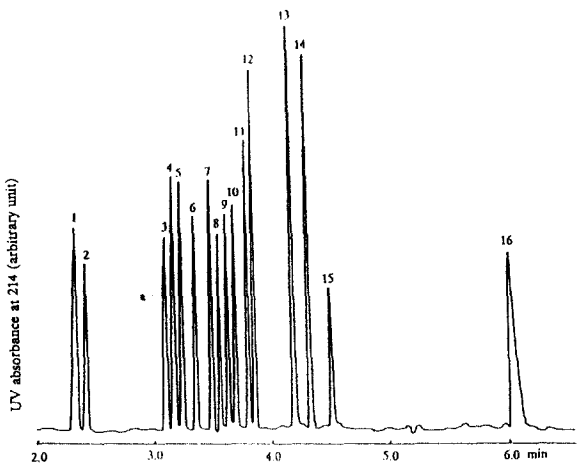

Fig. 1. Separation of 16 common metal ions and ammonium. Electrolyte, 11 m*M* lactic acid, 2.6 m*M* 18-crown-6, 7.5 m*M* 4-methylbenzylamine, 8% methanol, pH 4.3; applied voltage, 30 kV; injection time, 30 s. Peaks: 1 = NH_4^+ (5 ppm); 2 = K^+ (5 ppm); 3 = Na^+ (3 ppm); 4 = Ca^{2+} (3 ppm); 5 = Sr^{2+} (5 ppm); 6 = Mg^{2+} (1.5 ppm); 7 = Mn^{2+} (3.2 ppm); 8 = Ba^{2+} (5 ppm); 9 = Cd^{2+} (4 ppm); 10 = Fe^{2+} (3.2 ppm); 11 = Li^+ (0.8 ppm); 12 = Co^{2+} (3.2 ppm); 13 = Ni^{2+} (3.2 ppm); 14 = Zn^{2+} (3.2 ppm); 15 = Pb^{2+} (5 ppm); 16 = Cu^{2+} (4 ppm).

Table 1
Effect of 2.6 m*M* 18-crown-6 on the migration times (t_M) of metal ions in electrolyte also containing 11 m*M* lactic acid, 7.5 m*M* 4-methylbenzylamine and 8% methanol, buffered to pH 4.3

Ion	Lactic acid, t_M (min)	Lactic acid + 18-crown-6, t_M (min)	t_M increase (%)
NH_4^+	2.33	2.37	1.7
K^+	2.33	2.54	9.0
Ba^{2+}	2.78	3.75	35
Sr^{2+}	2.96	3.41	15
Na^+	3.05	3.14	3.0
Ca^{2+}	3.12	3.22	3.2
Mg^{2+}	3.28	3.43	4.6
Mn^{2+}	3.42	3.54	3.5
Cd^{2+}	3.55	3.68	3.7
Li^+	3.70	3.85	4.1
Co^{2+}	3.81	3.95	3.7
Pb^{2+}	4.08	4.81	18
Ni^{2+}	4.15	4.44	7.0
Zn^{2+}	4.30	4.62	7.4
Cu^{2+}	5.96	6.20	4.0

3.2. *Effect of methanol*

Several investigators have studied the effect of organic solvents on CZE [13,19–21]. Acetophenone was used as a neutral marker to measure the electroosmotic mobility as a function of methanol content in the electrolyte. The electroosmotic mobility decreases in a non-linear manner, as shown in Fig. 2. The electrophoretic mobility decreases almost linearly as the percentage of methanol in the electrolyte is increased (Fig. 3). The sum of these effects is a non-linear increase in migration times of the metal ions (Fig. 4). In general, methanol in the electrolyte improves the separation of metal ions having adjacent migration times.

3.3. *Effect of a high Na^+ concentration*

One of the shortcomings of CZE is that ion peaks become very broad at higher concentrations. We investigated the determination of 1 ppm each of Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} in the presence of 75 ppm of Na^+. Fig. 5 shows that

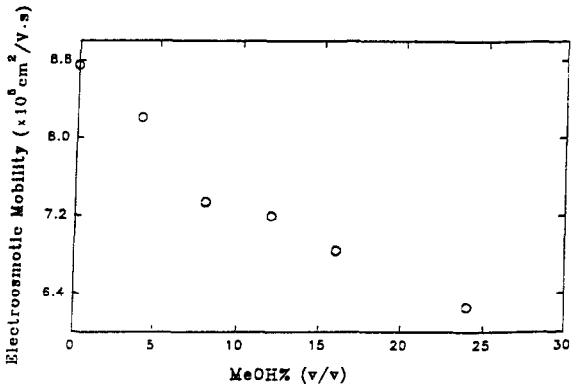

Fig. 2. Change of electroosmotic mobility with methanol as a buffer modifier. Electrolyte, 15 m*M* lactic acid, 10 m*M* 4-methylbenzylamine, 0–24% (v/v) methanol, pH 4.3; applied voltage, 20 kV; injection time, 30 s; neutral marker, acetophenone.

only Mg^{2+} can be separated from this concentration in 8% methanol, and only Mg^{2+} and Ca^{2+} can be determined in 16% methanol or 32% methanol. However if 18-crown-6 is added to the electrolyte, the migration times of Sr^{2+} and Ba^{2+} are slowed sufficiently that all four of the trace metal ions can be determined (Fig. 6). By lowering the applied voltage and reducing the injection time from 30 s to 6 s, 1 ppm of each of the four trace metal ions can be determined in the presence of 1000 ppm of sodium (Fig. 7).

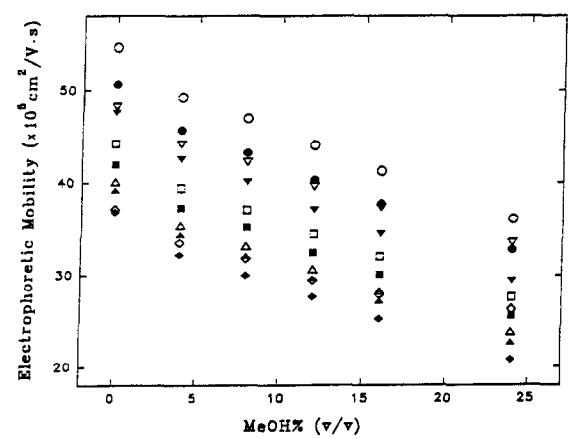

Fig. 3. Change of electrophoretic mobility with methanol as a buffer modifier. Conditions as in Fig. 2. ○ = Ba; ● = Sr; ▽ = Na; ▼ = Ca; □ = Mg; ■ = Mn; △ = Cd; ▲ = Fe; ◇ = Li; ◆ = Co.

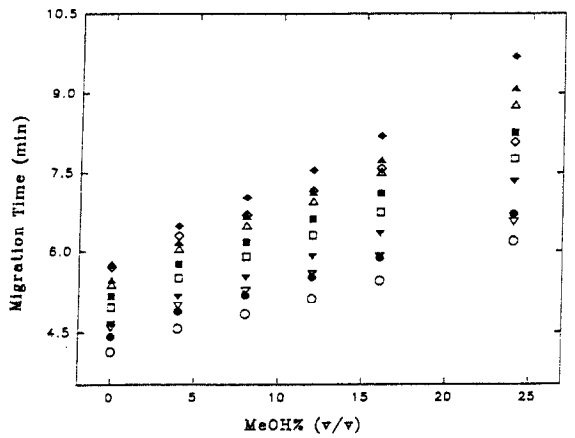

Fig. 4. Change of migration time with methanol as a buffer modifier. Conditions as in Fig. 2. Symbols as in Fig. 3.

3.4. Calibration plots

Known concentrations of each of the metal ions were separated under the conditions used in Fig. 1 to test the quantitative aspects. Lithium(I)

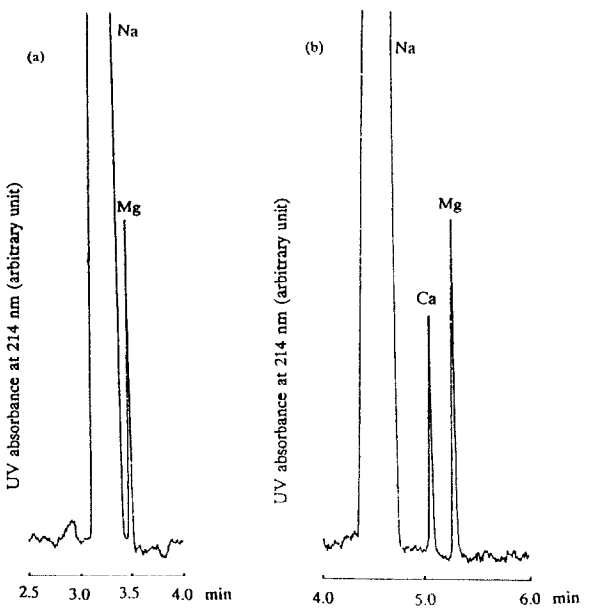

Fig. 5. Effect of methanol in the separation of 75 ppm Na^+ and 1 ppm Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+}. Electrolyte, 15 m*M* lactic acid, 10 m*M* 4-methylbenzylamine, pH 4.3, (a) 8% (v/v) methanol; (b) 32% (v/v) methanol; applied voltage, 30 kV; injection time, 30 s.

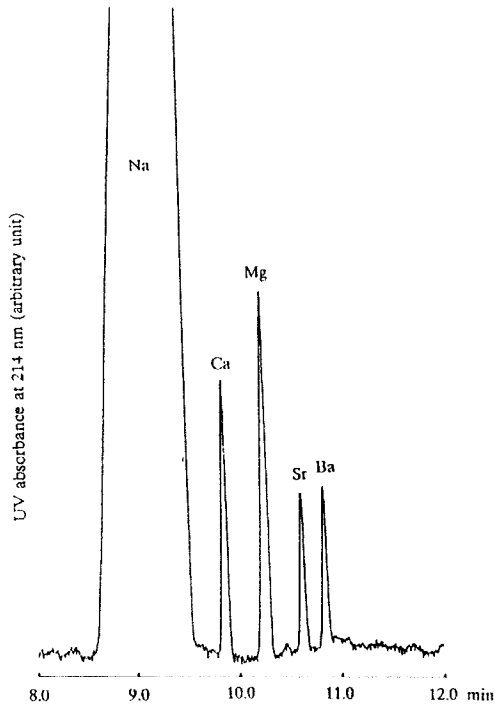

Fig. 6. Effect of 18-crown-6 in the separation of 75 ppm Na^+ and 1 ppm Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+}. Electrolyte, 15 m*M* lactic acid, 10 m*M* 4-methylbenzylamine, 32% (v/v) methanol, 3.0 m*M* 18-crown-6, pH 4.3; applied voltage, 15 kV; injection time, 30 s.

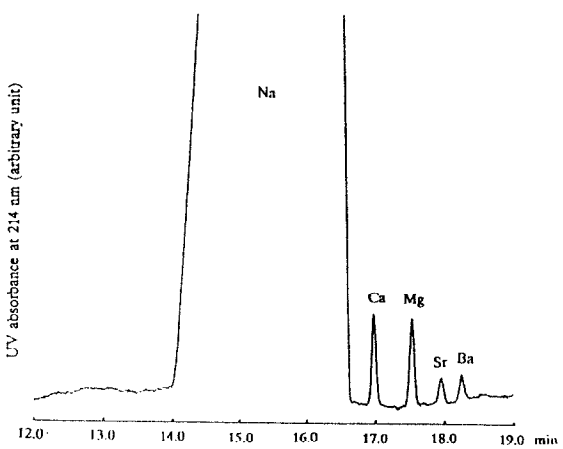

Fig. 7. Separation of 1 ppm Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} from 1000 ppm Na^+. Electrolyte conditions same as Fig. 6; applied voltage, 10 kV; injection time, 6 s.

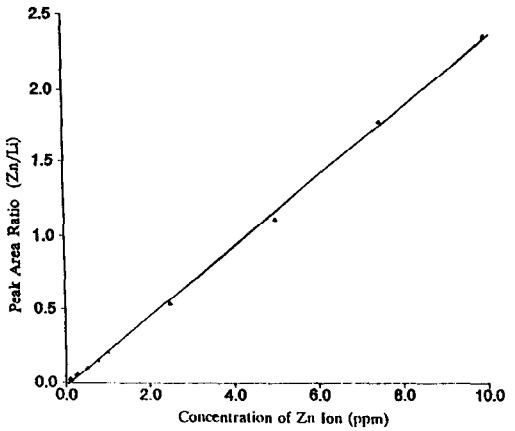

Fig. 8. Calibration plot of Zn^{2+} using Li^{+} as an internal standard. Electrolyte, 15 m*M* lactic acid, 10 m*M* 4-methylbenzylamine, 0.6 m*M* 18-crown-6, 10% (v/v) methanol, pH 4.3; applied voltage, 20 kV; injection time changing with the concentration of Zn^{2+} standard solution, 60 s for 0.10 ppm and 0.25 ppm, 30 s for 0.50 ppm to 2.5 ppm, 15 s for 5.0 ppm, 15 s for 5.0 ppm, 11 s for 7.5 ppm, 8 s for 10.0 ppm; internal standard concentration, 0.2 ppm Li^{+} in 0.10 ppm or 0.25 ppm Zn^{2+} solution, 1.0 ppm Li^{+} in 0.5 or higher concentration of Zn^{2+} solution.

was selected as an internal standard. As might be expected plots of peak height against concentrations were unsatisfactory over the range of 1 to 10 ppm. However, a plot of peak area (relative to that of Li^{+}) *vs.* concentration gave a linear plot over a 100-fold change in concentration (0.1 to 10 ppm). A typical plot (Fig. 8) shows linearity over the entire range. Slopes and correlation coefficients are listed in Table 2.

Table 2
Slopes and correlation coefficients of calibration plot for quantitative analysis of several metal cations

Ion	Slope (1/ppm)	Correlation coefficient
Sr^{2+}	0.159	0.9983
Mg^{2+}	0.560	0.9992
Mn^{2+}	0.241	0.9989
Co^{2+}	0.277	0.9995
Zn^{2+}	0.236	0.9995

Electrolyte, 15 m*M* lactic acid, 10 m*M* 4-methylbenzylamine, 0.6 m*M* 18-crown-6, 10% MeOH, pH 4.3; applied voltage, 20 kV; injection time, 8–60 s. The concentration of all metal ion standards is from 0.1 ppm to 10.0 ppm.

3.5. Separation efficiency

The electrophoretic peaks were examined with a recorder chart speed of 10 cm/min to spread out the individual peaks and facilitate measurement of peak width. The plate number (N) widely used in chromatography was calculated for Zn^{2+}, which was one of the better shaped peaks. Mikkers *et al.* [22,23] have previously explained why peak efficiency in CZE decreases with increasing sample load. At 1 ppm, the calculated value of N was *ca.* 365 000, which would indicate very efficient electrophoretic behavior. However, the calculated plate number dropped drastically as the amount of Zn^{2+} injected was increased (Fig. 9). A somewhat similar decrease in plate number with increasing amounts of sample ions was noted in a recent paper [3].

3.6. Separation of uncomplexed metal cations

Hydroxyisobutyric acid (HIBA) and lactic acid have been used extensively to separate groups of metal ions that have almost identical mobilities, such as the divalent transition metal ions and the trivalent lanthanides. Metal ions are complexed to varying degrees, resulting in differences in the

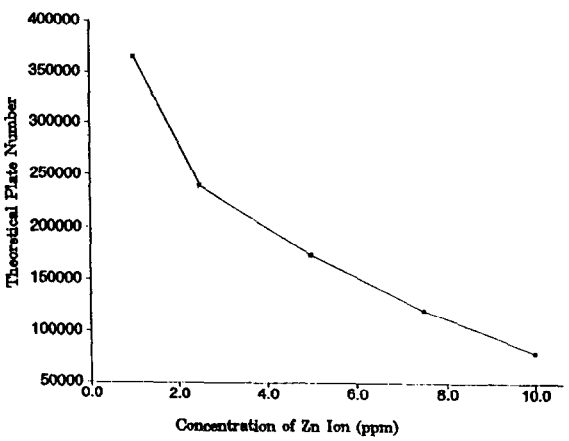

Fig. 9. Change of theoretical plate number with analyte concentration. Same electrolyte condition and applied voltage as described in Fig. 8; injection time, 15 s. Theoretical plate number calculated using $(t_M/\sigma)^2$, where t_M = the migration time and $\sigma = 2.35\ W_{1/2}$ which is the half width of a peak.

overall rates of movement of the various metal ions. The zone of each element contains the free metal cation in equilibrium with one or more metal-ligand species. In the separation of the lanthanides with lactic acid as the ligand, the migration time has been shown to be a linear function of $\bar{n}$, the average number of ligands attached to the lanthanide [13]. It will be seen that the equilibria between the free metal ion and the several complexed species must be quite fast in order to maintain a tight, compact zone. If the equilibria are not rapid, the different species would move at different rates and the zone for an element would become very diffuse.

In the lactic acid system no peak could be obtained for ions such as Al(III) and Fe(III). This could be due to their complexation kinetics. We therefore decided to study the separation of metal ions by CZE under conditions where only free, uncomplexed ions would be present. To avoid complications from hydrolysis of metal ions, a very acidic pH was chosen.

Attempts were made to separate metal ions such as Cu^{2+} and Cr^{3+}, which have a reasonable absorbance in the UV spectral range. Using buffers such like protonated β-alanine (pK_a = 3.6)–β-alanine or formic acid (pK_a = 3.75)–formate, Cu^{2+}, Cr^{3+}, UO_2^{2+} and VO^{2+} were separated and directly detected at 214 nm. However, the detection sensitivity was rather poor. The absorbance of many other metal ions is so low that direct detection is not feasible. The situation becomes even worse if only a few detection wavelengths are available on the instrument.

For these reasons indirect detection is the better choice for multi-element detection with high sensitivity. In practice, not all buffer systems are suitable for the indirect detection. The baseline was very noisy and no metal ion peaks could be discerned with β-alanine or formate as the buffer and 4-methylbenzylamine or phenylethylamine as the UV-visualizing agent. However, good peaks were obtained at pH 3.2 with nicotinamide (pK_a = 3.3) as the UV-visualizing coion, and formate as the counterion.

Fig. 10a shows a separation of several metal ions at pH 3.2 using 8.0 m*M* nicotinamide–formate buffer. It is now possible to obtain good

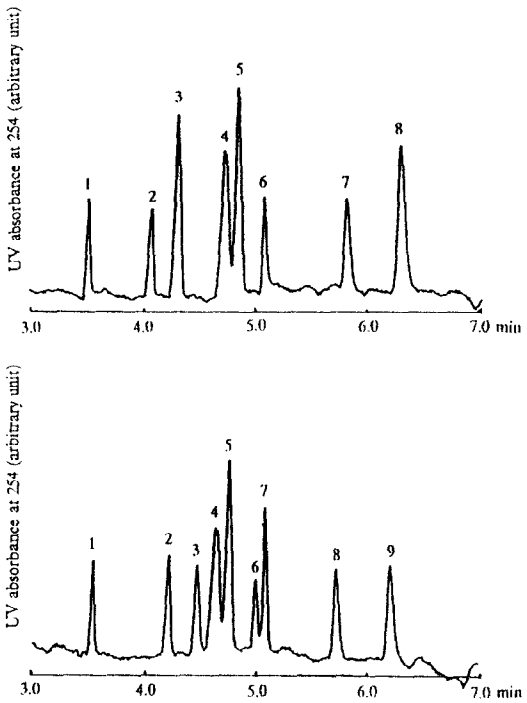

Fig. 10. (a) Separation of a sample standard mixture using an uncomplexing electrolyte. Electrolyte, 8 m*M* nicotinamide, pH 3.2 adjusted with formic acid; applied voltage, 25 kV; injection time, 40 s. Peaks: 1 = K^+ (1.5 ppm); 2 = Ba^{2+} (1.5 ppm); 3 = Sr^{2+} (1.5 ppm) and Ca^{2+} (0.8 ppm); 4 = Mg^{2+} (0.5 ppm) and Na^+ (0.8 ppm); 5 = Al^{3+} (0.8 ppm); 6 = Cu^{2+} (0.8 ppm); 7 = Li^+ (0.2 ppm); 8 = VO^{2+} (2.0 ppm). (b) Separation of the same sample standard mixture using an uncomplexing electrolyte with 18-crown-6. Electrolyte, 8 m*M* nicotinamide, 0.6 m*M* 18-crown-6, pH 3.2 adjusted with formic acid; applied voltage, 25 kV; injection time, 40 s. Peaks: 1 = K^+ (1.5 ppm); 2 = Ca^{2+} (0.8 ppm); 3 = Sr^{2+} (1.5 ppm); 4 = Mg^{2+} (0.5 ppm) and Na^+ (0.8 ppm); 5 = Al^{3+} (0.8 ppm); 6 = Cu^{2+} (0.8 ppm); 7 = Ba^{2+} (1.5 ppm); 8 = Li^+ (0.2 ppm); 0 = VO^{2+} (2.0 ppm).

peaks for Al^{3+} and VO^{2+}. In other experiments good peaks were obtained for UO_2^{2+}, Cr^{3+} and Ag^+.

In Fig. 10a Ca^{2+} and Sr^{2+} co-migrate (peak 3) and Mg^{2+} and Na^+ co-migrate in peak 4. The separation was repeated under the same conditions but with 0.6 m*M* 18-crown-6 also added to the electrolyte (Fig. 10b). The crown ether lengthened the migration times of Sr^{2+} and Ba^{2+} so that separation of Ca^{2+} and Sr^{2+} was obtained.

The separation of Al^{3+} was tried under the

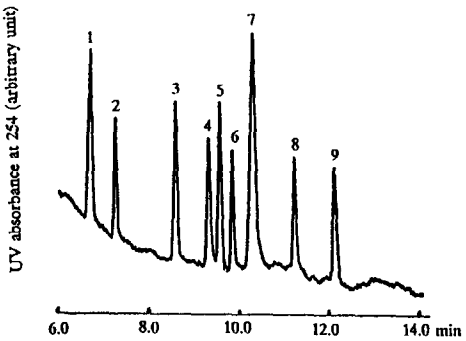

Fig. 11. Electropherogram of a standard mixture with nine common cations using a nicotinamide electrolyte. Electrolyte, 8 m*M* nicotinamide, 12% methanol, 0.95 m*M* 18-crown-6, pH 3.2 adjusted with formic acid; applied voltage, 25 kV; injection time, 40 s. Peaks: 1 = NH_4^+ (1.5 ppm); 2 = K^+ (1.5 ppm); 3 = Ca^{2+} (1.0 ppm); 4 = Na^+ (1.0 ppm); 5 = Mg^{2+} (0.5 ppm); 6 = Sr^{2+} (1.0 ppm); 7 = Al^{3+} (1.0 ppm); 8 = Ba^{2+} (1.0 ppm); 9 = Li^+ (0.2 ppm).

same conditions as used for the separation shown in Fig. 10b but with lactic acid added to the electrolyte. No Al^{3+} peak was found, suggesting that the kinetics of forming and dissociating the Al^{3+}–lactate complex may be slow. The crown ether in the electrolyte probably does not complex Al^{3+}.

By adjusting slightly the concentrations of nicotinamide and crown ether, and by adding methanol to the electrolyte, it was possible to completely resolve a mixture containing Mg^{2+}, Al^{3+}, the alkaline earths and the first three alkali metal ions (Fig. 11).

4. Acknowledgements

We wish to thank Waters Division of Millipore for their gift of the Waters Quanta 4000 CE system and associated chemicals. We also thank Andrea Weston and Peter Jandik for valuable technical assistance in the initial stages of this research.

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