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Capillary electrophoretic analysis of inorganic cations

Role of complexing agent and buffer pH

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ABSTRACT

Capillary electrophoresis for the determination of inorganic metal cations in the presence of various complexing agents was investigated. The complexing agents studied were acetic, glycolic, lactic, hydroxyisobutyric, oxalic, malonic, malic, tartaric, succinic and citric acid. They were all suitable as complexing agents for separating a mixture of six alkali and alkaline earth metal ions (lithium, sodium, potassium, magnesium, calcium and barium) using indirect UV detection with imidazole as a carrier buffer and background absorbance provider. The pH of the carrier buffer affected the electrophoretic separation in a complex but predictable way. The optimum pH for separating these ions in the presence of the complexing agent was around the pK_1 of the acid. When di- and triprotic acids were used, electrophoresis carried out above the second acid dissociation constant resulted in a significant decrease in the mobility of divalent ions and a decrease in number of theoretical plates, N, due to complex formation. In most of the cases reported here one could obtain, typically, a migration time span from 1 to 2 min, a minimum and a maximum resolution of 1 and 15, respectively, and N from 16 000 to 750 000 per metre. Of the ten complexing agents studied, lactic, succinic, hydroxyisobutyric and malonic acid seemed to give the best overall performance.

INTRODUCTION

Capillary electrophoresis (CE) has rapidly developed into a reliable microanalytical separation technique for a variety of applications [1,2]. Inorganic ion analysis, however, has received relatively little attention. This is due, in part, to the existence of other sensitive techniques and the lack of a direct detection method. The latter problem has been solved recently by the introduction of indirect UV absorption method [3–7], which is readily available with all commercial capillary electrophoresis systems.

Foret *et al.* [3] utilized indirect UV detection to demonstrate the separation of fourteen lanthanide cations by CE. Highly efficient separation was obtained within 5 min with the aid of hydroxyisobutyric acid (HIBA) as a complexing agent and creatinine as a UV-absorbing co-ion. Wildman *et al.* [4] and Weston and co-workers [5–7] investigated the factors that affect the separation of metal cations and optimized the detection sensitivity for metal cations using indirect photometric detection. They showed that a mixture of nineteen alkali, alkaline earth and lanthanide metal cations can be resolved at the baseline level within less than 2 min. Beck and Engelhardt [8] investigated several background electrolytes for indirect UV detection and found imidazole to be suitable for separation of metal ions, amines and amino alcohols. Gross and Yeung [9] employed indirect fluorescence detection for the CE of several metal cations. Bachmann *et al.* [10] utilized Ce(III) as a fluorescent carrier electrolyte and reported indirect fluores-

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cence detection for the CE of ammonium and alkali and alkaline earth metal ions. Swaile and Sepaniak [11] described the separation of three divalent cations with a chelating and fluorescent agent, 8-hydroxyquinoline-5-sulphonic acid (HQS). Timerbaev *et al.* [12] used HQS for the CE of transition and alkaline earth metals as precolumn-formed chelates with direct UV detection. Aguilar *et al.* [13] presented a detection scheme for iron, copper and zinc cations in electroplating solution using cyanide complexes with direct UV detection.

In this study, we investigated the use of mono-, di- and triprotic carboxylic and hydroxycarboxylic acids as complexing agents in the separation of metal ions using the alkali and alkaline earth metal ions Li, Na, K, Mg, Ca and Ba as examples (charges are omitted for brevity). We have also studied how the pH of the carrier buffer affected the separation of these metal ions. These complexing agents can selectively modulate the mobility of metal cations by forming metal chelates with varying degrees of stability. It appears that the complexing agent and the pH of the carrier electrolyte influence the separation of these cations in a very intricate manner. They affect the electrophoretic mobility and the migration order of these ions, the separation efficiency as measured by the number of theoretical plates and the resolution.

EXPERIMENTAL

Chemicals

All metal ion solutions were prepared from chloride salts. They were prepared as stock solutions of 100 ppm, mixed and diluted to 10 ppm (metal ion concentration). Unless specified otherwise, all six ions shown in Figs. 1–6 were present at a concentration of 10 ppm each. The corresponding ion molar equivalent concentrations were Li 1.43, Na 0.44, Mg 0.41, K 0.26, Ca 0.25 and Ba 0.073 mequiv. Imidazole was used both as the background carrier electrolyte (providing strong UV absorption at 215 nm) and buffer ($pK_a = 6.95$). Ten carboxylic and hydroxycarboxylic acids were used (see Table I). They include monoprotic acetic, glycolic, lactic and hydroxyisobutyric acid, diprotic oxalic, malonic, succinic, malic and tartaric acid and triprotic citric acid. These acids and metal chloride salts and imidazole all were of analytical-reagent or reagent grade from various vendors. Doubly deionized water prepared with a Milli-Q system (Millipore, Bedford, MA, USA) or doubly deionized, distilled water was used exclusively for all solutions. The water blank was routinely checked for contamination with traces of alkali and alkali earth metal ions.

Buffers and pH adjustment

The running buffer contained 5 m*M* imidazole, which served as both the carrier electrolyte and background absorber for indirect UV detection. The pH was varied as specified in the figures, being adjusted by adding a 1 *M* stock solution of complexing agent to the desired pH in the range 3–6 depending on the experiments. The concentration of the complexing acid was varied from 0.1 to 6.4 m*M* (calculated by the volume added) as specified in the figures. For each acid studied, typically CE for each sample was carried out at 0.5 pH increments. When a fixed concentration of the complexing agent was desired, the pH of the solution was adjusted with aliquots of 1 *M* HCl (adjustment with sulphuric acid gave very different migration orders; see Results and Discussion).

Apparatus

CE experiments were carried out in a fully automated Spectra Phoresis Model 1000 instrument (Spectra Physics Analytical, San Jose, CA, USA). The system was equipped with a rapid-scanning UV–Vis detector with 5-nm wavelength resolution. In most of the cases reported here, however, the detector wavelength was fixed at 215 nm to obtain a more stable and less noisy baseline. The instrument was also equipped with autosamplers, a capillary cartridge and a solid-state Peltier temperature control unit. A personal computer (486 IBM AT-compatible PC) was used to control the instrument settings, data acquisition and analysis with the vendor-provided software. The separation capillaries (bare fused silica) from Polymicro Technologies (Phoenix, AZ, USA) were 42 cm (35 cm to the detector) × 50 μm I.D. × 375 μm O.D. UV–Vis

absorption spectra of the background electrolytes and complexing agents were measured with a Hitachi (Tokyo, Japan) U-2000 double-beam scanning spectrophotometer.

Electrophoretic procedures

Prior to first use, a new capillary was subjected to a wash cycle of 10 min each with the following steps: (a) 1 *M* NaOH, (b) 0.1 *M* NaOH and (c) deionized water at 60°C, (d) the running buffer at the running temperature and (e) the running buffer at the running voltage and temperature. Subsequent runs were carried out with the following standard cycle: (a) prefilled running buffer for 5 min, (b) sample injection, (c) separation run at the indicated voltage and temperature and (d) deionized water post-wash for 3 min. Sample injection was carried out in the electrokinetic mode at +5 kV for 2 s. The separation run was at +25 kV constant voltage at 25°C constant temperature and with a current of 2–10 μA. The capillary was also washed with 0.1 *M* NaOH and deionized water as a daily routine. All buffer solutions were freshly prepared using deionized, distilled or doubly deionized water, filtered through 0.20-μm membranes and degassed under vacuum for 10 min.

RESULTS AND DISCUSSION

Influence of complexing agents on migration time and order

As reported previously [8], the separation was most efficient if CE was carried out when the mobility of the analytes matched well that of the carrier electrolyte. Imidazole has been found to be satisfactory for this purpose. However, in the absence of a complexing agent, only five peaks were found (Fig. 1a); Na and Mg could not be resolved. Changing the pH of the buffer changed only the migration time span [the separation time was shorter at higher pH owing to the increase in the electroosmotic flow (EOF)]; it could not resolve the Na–Mg peak or alter the migration order. In general, the migration time was shorter in the absence of a complexing agent at the same pH. We also noted that the acid added to adjust the buffer pH affected the resolution and the migration order of the ions, in particular for Ba, Mg and Ca. If HCl was used to adjust the pH, Na and Mg could not be resolved. If H_2SO_4 was used instead, then all six ions could be separated in the absence of a complexing agent; the migration order was Na < Ba < Ca, as found previously [8].

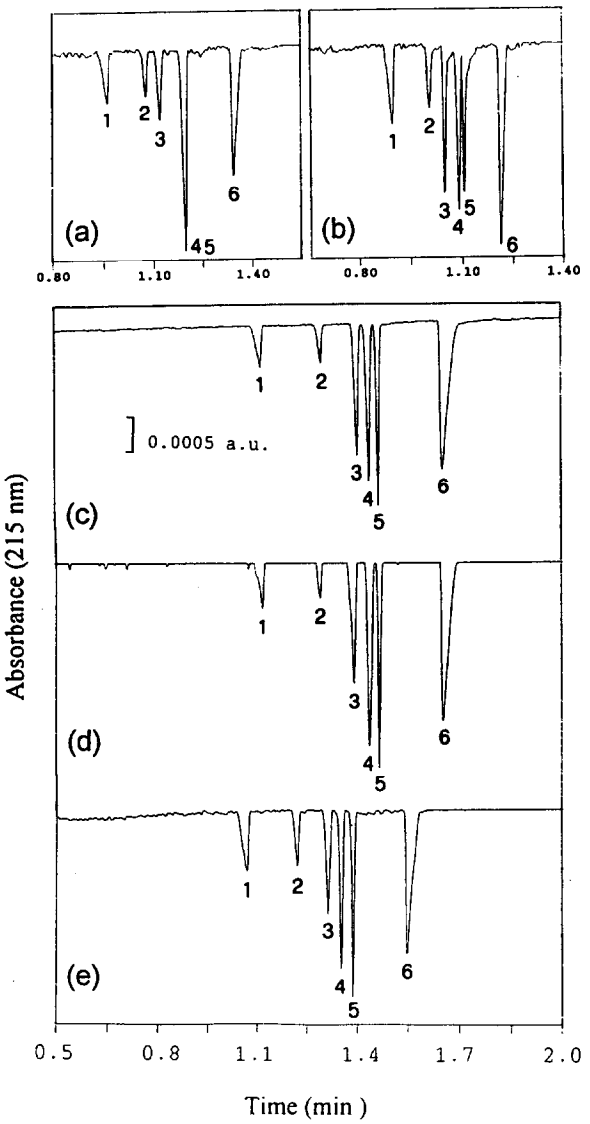

Fig. 1. Comparison of electropherograms (a) in the absence of complexing agent (pH 4.0) and (b) in the presence of 2.1 m*M* acetic acid (pH 6.0), (c) 4.55 m*M* glycolic acid (pH 4.0), (d) 5.0 m*M* lactic acid (pH 4.0) and (e) 6.4 m*M* HIBA (pH 4.0). Peaks K, Ba, Ca, Na, Mg and Li in that order in all figures. The pH was adjusted with (a) 1 *M* HCl and (b)–(e) a 1 *M* solution of the complexing acids.

Monoprotic acids. The four monoprotic complexing agents, acetic, glycolic and lactic acid and HIBA, affected the separation of the six metal ions in a similar way. The pK_a values for these four acids are given in Table I. Note that the hydroxy group lowers the pK_a of comparable carboxylic acids. As with HIBA reported previously [6–8], separation into six peaks could be readily seen in the presence of any of the other three monoprotic acids. The migration order of the six ions, K < Ba < Ca < Na < Mg < Li, was the same in these four acids. The electropherograms in the absence of complexing agent and in the presence of these four monoprotic acids are compared in Fig. 1a–e. The migration order was not altered significantly by the pH but the resolution at the baseline level was influenced by the pH. The pH range where the migration order was not affected by the pH and a good separation could be obtained was 3.5–6.0 for lactic acid, 3.5–5.0 for glycolic acid, 3.5–5.5 for HIBA and 6.0–6.5 for acetic acid. Note that in acetic acid a baseline separation of all ions was possible only at pH > 6.0. The migration time and its span were shorter at higher pH owing to the increase in EOF, as expected. All four monoprotic acids are suitable as complexing agents in the pH range indicated above. As the pK_a of the carrier electrolyte imidazole is about 6.9, the CE operating pH is restricted to about 6.0. However, as noted above, adjustment of the pH with H_2SO_4 should be avoided. In HIBA, we found that if the pH was adjusted to 4.5 with H_2SO_4, the Ca and Na peaks could not be resolved (Fig. 2a). However, lowering the pH to 4.0 (Fig. 2b)

TABLE I

pK_a VALUES OF THE VARIOUS COMPLEXING AGENTS

Value for HIBA from ref. 14, all others from ref. 15.

Acid	pK_a	Acid	pK_a
Acetic	4.75	Malonic	2.83, 5.69
Glycolic	3.83	Succinic	4.16, 5.61
Lactic	3.86	Malic	3.40, 5.11
HIBA	3.97	Tartaric	2.98, 4.34
Oxalic	1.23, 4.19	Citrate	3.14, 4.77, 6.39

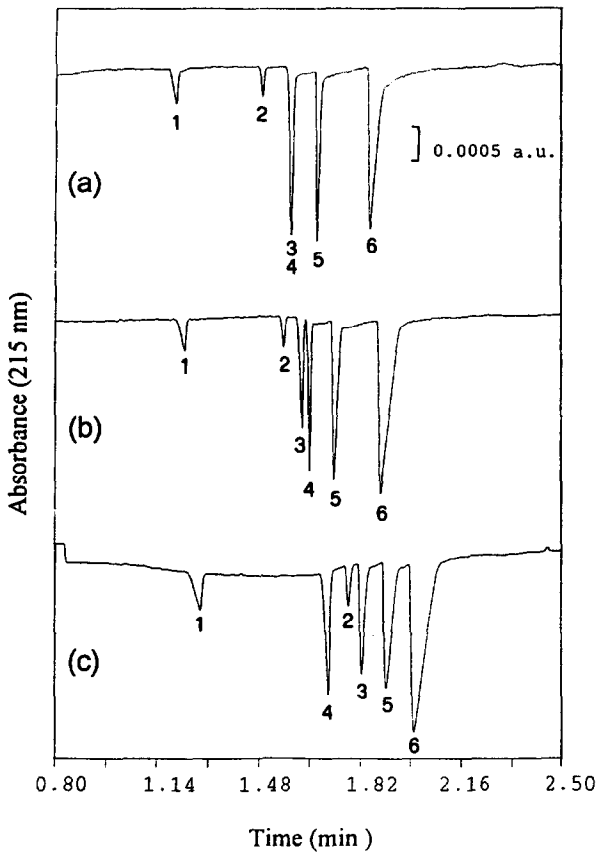

Fig. 2. Effect of pH adjusted with sulphuric acid on the electropherograms in the presence of 3 m*M* HIBA at pH (a) 4.5, (b) 4.0 and (c) 3.0. Peaks as in Fig. 1.

restored the migration profile as in HIBA without H_2SO_4 (Fig. 1e). Adjusting the pH down to 3.0 with H_2SO_4 changed the migration order completely; all three divalent ions (peaks 2, 3 and 5) migrated more slowly and were between Na and Li (peaks 4 and 6, Fig. 2c). All six peaks seemed to be broadened considerably. It seems that sulphate anion behaves similarly to a complexing agent and affects the migration of divalent ions even more than HIBA.

Diprotic acids. The effects of diprotic oxalic, malonic, succinic, malic and tartaric acid on the separation of metal ions were more complex, depending also on the pH. The acid dissociation constants, pK_1 and pK_2, of these acids are also given in Table I. A dicarboxylic acid with a longer carbon chain has higher acid dissociation constants. A hydroxyl group lowers the first and

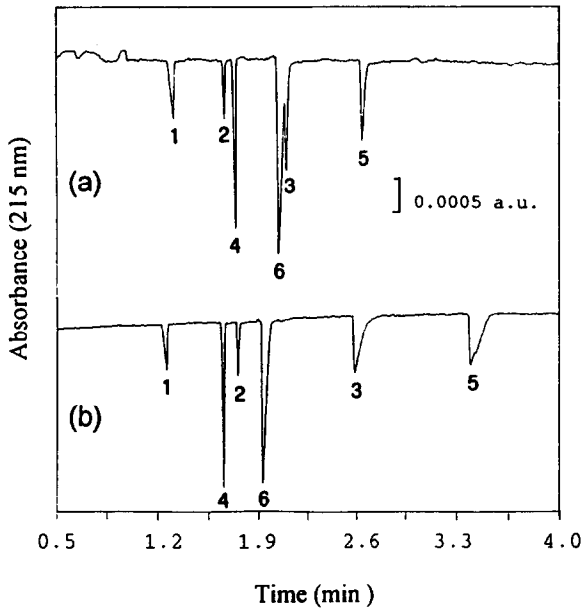

Fig. 3. Comparison of electropherograms in the presence of oxalic acid at (a) 3.4 m*M* (pH 3.0) and (b) 1.9 m*M* (pH 4.0). The pH was adjusted with 1 *M* oxalic acid. Peaks as in Fig. 1.

second acid constants by about 0.5–0.6 p*K* unit. In oxalic acid at pH 3.0 (Fig. 3a), the resolution of Li and Ca was poor. The migration order of the six ions was considerably different from that in the monoprotic acids. The pH affected the migration time and order and also the resolution substantially. At pH 4.0 (Fig. 3b), all ions were well separated. The mobilities of all three divalent ions decreased greatly, resulting in a much larger time span. The migration order also changed; Ba migrated behind Na, and Ca and Mg both moved far behind Li. Apparently, this was due to the complex formation of divalent ions with the anionic conjugated base. Using oxalic acid as the complexing agent, the pH range suitable for the CE is 4.0–6.0.

The best separation in the presence of malonic acid was obtained at pH 4.0 (Fig. 4a). All six ions were well separated at the baseline level and the migration order was the same as with the monoprotic acids. At higher pH, the degree of complexing of the divalent cations with malonic acid seemed to increase, resulted in overlap of the Ba peak (at pH 5.5, Fig. 4b) or the Ca peak (pH 5.0, data not shown) with the Na peak. In

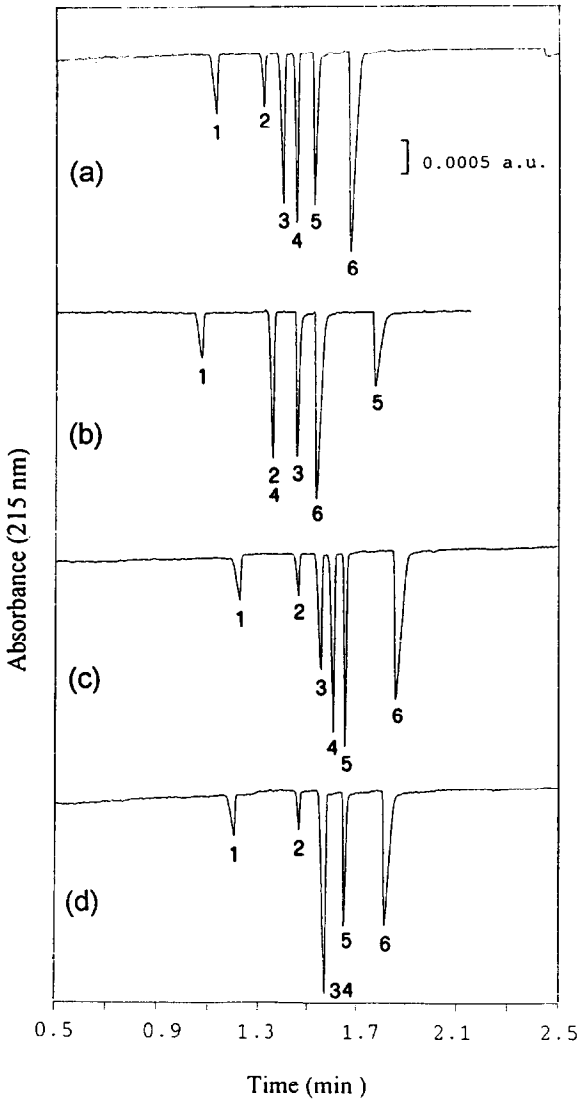

Fig. 4. Comparison of electropherograms in the presence of malonic acid at (a) 4.0 m*M* (pH 4.0) and (b) 2.2 m*M* (pH 5.5) and in the presence of succinic acid at (c) 3.0 m*M* (pH 4.5) and (d) 2.1 m*M* (pH 5.0). The pH was adjusted with a 1 *M* solution of the complexing acid. Peaks as in Fig. 1.

succinic acid, the effect of pH on the separation of the ions was similar to that in malonic acid. The best separation was obtained at pH 4.5 with the same migration order as in malonic acid (Fig. 4c). The pH affected the migration profile in a similar way. As the pH increased, Ca migrated more slowly and overlapped the Na peak at pH 5.0 (Fig. 4d) but moved apart again at pH 5.5 (thus the Ca peak moved behind the Na peak;

data not shown). Comparing the electrophoretic profiles in these three acids, the separation of ions in malonic and succinic acid was more equally spaced and in a narrower time span than in oxalic acid. As the divalent cations formed a more stable ring–chelate complex with oxalate than with malonate or succinate, the complexing effect in oxalic acid seemed to be more pronounced. Also the effect of pH, which affected the degree of ionization of the complexing acid (and thus the stability of the metal chelate), on the separation of the ions seemed to be more sensitive with oxalate.

In malic acid, a baseline-resolved separation into six peaks was possible only at pH ≈ 3.7 (Fig. 5a). At higher pH, all three divalent cations seemed to form complexes with malate anion and migrated more slowly (Fig. 5b). The migration order depended greatly on the pH, which affected mostly the mobilities of the three divalent ions. Similarly, in tartaric acid baseline separation into six peaks could be obtained only at pH 4.0 (Fig. 5c). Also similarly to malic acid, as the pH was raised to 5.0 all three divalent ions migrated more slowly, the Ba and Ca peaks merged and moved closer to the Li peak and the Mg peak appeared further away from the Li peak (Fig. 5d). The two hydroxy diprotic acids behaved differently to the above three diprotic acids. The pH effect in the two hydroxy acids seemed to be more sensitive and pronounced, resulting in a very limited pH range where all six ions could be baseline resolved.

Triprotic acid. The effect of triprotic citric acid on the separation of divalent ions was even greater than that of diprotic acids. As expected, the influence was dependent on the pH, which determined the charge carried by the metal chelate complex. The stability constants of citrate with all three alkali earth metal ions are high [14]. Hence the experiments were carried out at different citrate concentrations. A baseline separation of all six ions could be obtained at pH 4.5 (adjusted with citric acid to 2.4 m*M*). All three divalent ions trailed behind the alkali metal ions (Fig. 6a) with a large time span. Both Ca and Mg, which migrated far behind the Ba peak, showed a considerable triangular trailing shape. If the concentration of

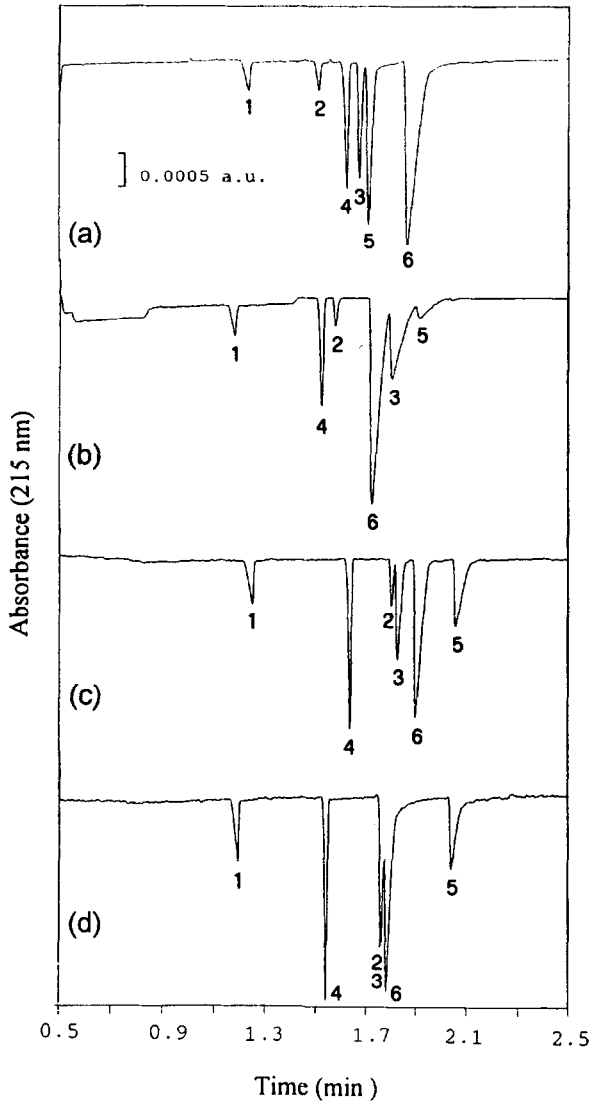

Fig. 5. Comparison of electropherograms in the presence of malic acid at (a) 3.6 m*M* (pH 3.7) and (b) 1.8 m*M* (pH 5.0) and in the presence of tartaric acid at (c) 1.9 m*M* (pH 4.0) and (d) 1.4 m*M* (pH 5.0). The pH was adjusted with a 1 *M* solution of the complexing acid. Peaks as in Fig. 1.

citric acid was kept at 1 m*M* and the pH was adjusted to 3.0–4.0 with 1 *M* HCl, the migration order was very different (Fig. 6b). At pH 3.0 hardly any complexes seemed to be formed and Ba and Ca migrated ahead of Na, which co-migrated with Mg. At pH 3.5 Ca co-migrated with Na but Mg was separated (data not shown). However, no complete separation of all six ions

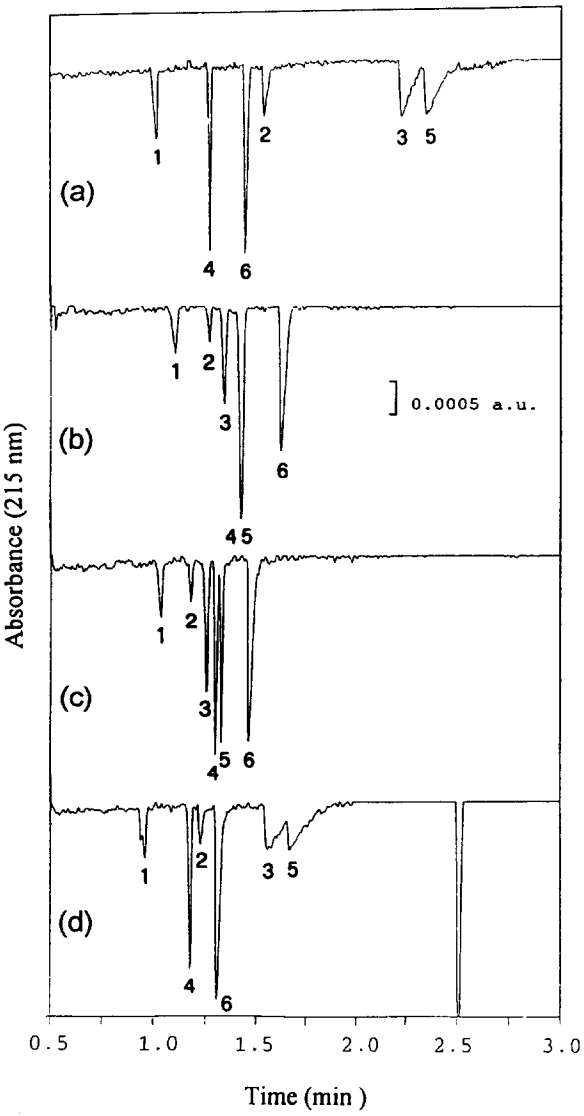

Fig. 6. Effect of citrate concentration and pH on the electropherograms. Citrate concentration: (a) 2.4; (b) 1.0; (c) and (d) 0.1 m*M*. pH: (a) 4.5; (b) 3.0; (c) 4.0; (d) 5.5. The pH was adjusted with (a) 1 *M* citric acid and (b)–(d) 1 *M* HCl. Peaks as in Fig. 1.

could be obtained under these conditions. When the citrate concentration was lowered further to 0.1 m*M*, a marginal separation of all six ions could be obtained at pH 4.0 (Fig. 6c) with six sharp peaks similar to those observed in mono- or diprotic acids. As the pH increased, Ba, Ca and Mg ions all moved more slowly. Their peaks overlapped with the Na and Li peaks, depending on the pH used. At pH 5.5 the electrophoretic profile became very similar to that at higher citrate concentration (Fig. 6d). Thus, in citric acid, the separation was affected both by the concentration of the complexing agent and the pH. Fig. 7 shows a comparison of migration time and order for the six ions in these ten acids at the pH were optimum separation can be obtained. While critic and oxalic acid have a larger separation time span, all eight other acids provide faster separation with reasonable to excellent baseline separation. Further, the first four acids provide a wider operating pH range.

Resolution and number of theoretical plates

The resolution was judged by the number of theoretical plates, N, and maximum and minimum peak separation, R_s. These parameters, analogous to those used in the liquid chromatography, are calculated by the following equation for each ion:

$$N = 5.54(t_m/W_{1/2})^2$$

$$R_s = 2[(t_m)_2 - (t_m)_1]/(W_1 + W_2)$$

where t_m, $W_{1/2}$ and W are the migration time, the width of the peak of the ion at half-height and

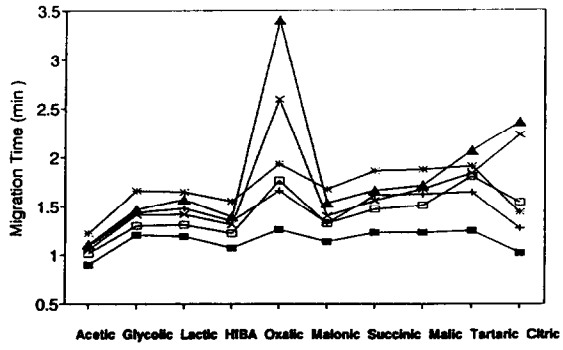

Fig. 7. Comparison of migration times for the six ions in the presence of different complexing agents in the pH range where optimum separation could be obtained: in acetic acid at pH 6.0, in succinic and citric acid at pH 4.5, in malic acid at pH 3.7 and in all other acids at pH 4.0. The pH was adjusted with a 1 *M* solution of the complexing acid. ■ = K; + = Na, * = Li; □ = Ba; × = Ca; ▲ = Mg.

the width of the peak of the ion at the baseline in the electrophortogram, respectively; subscripts 1 and 2 refer to the peak number. The influence of complexing agents on the resolution of electropherograms is compared in Fig. 8. In general, the ions that co-migrate closer to the carrier imidazole have better N values. Thus, the fronting K and the trailing Li both have poorer N values than the other ions and are affected less by the type of acid present. The N values for Ca and Mg varied most significantly; they are particularly low in oxalic and critic acid, in which the stability constants of the complexing agent with these two ions is high. In citric acid, Na has the best N, but all other divalent ions have the poorest N. In glycolic acid, Mg has the highest N of all, reaching 760 000/m.

The ion pair with the largest peak separation distance is K–Li with the maximum R_s of 8.4–15.9 in most of the complexing agents except tartaric, oxalic and citric acid. In these three acids, K–Mg has the maximum R_s of 12.3–22.9. The minimum R_s varies in different acids; the Na–Ca pair ($R_s = 0.7$–1.2) is usually more difficult to separate than any other ion pairs.

Reproducibility, quantification, linearity and detection limit

The reproducibility of the CE method was studied by making five consecutive runs with all six ions present at 10 ppm in 3.0 mM succinic acid–5 mM imidazole. All other electrophoretic conditions were the same except that hydrodynamic (HD) sample injection for 2 s was also studied in addition to the standard electrokinetic injection (EK) mode (+5 kV for 2 s). The precisions in terms of relative standard deviations (R.S.D.) for the EK and ED modes are compared in Table II. Both injection modes provided excellent precision for the migration time but not as good for the peak area. The better peak-area precision for Li could be attributed to the higher molar equivalent concentration of Li ions present in the mixture. For quantitative purpose, it may be better to use peak height, which offered a slightly better precision. On the other hand the calculated percentage peak-area ratio for K:Ba:Ca:Na:Mg:Li, being 4.1:2.5:9.9:10.5:20:53, is more consistent with the molar equivalent concentration ratio of these ions present in the standard solution. The peak-height ratio is not in the correct proportion to the molar equivalent concentration for Mg and Li, as is apparent in all the figures shown. This is due in part to the broader peak width and more significant trailing of the Li peak.

The calibration graphs expressed as peak area *vs.* concentration in the concentration range 0.0145–1.45 mequiv. (0.1–10 ppm for Li) are compared in Fig. 9. All five ions (K was not

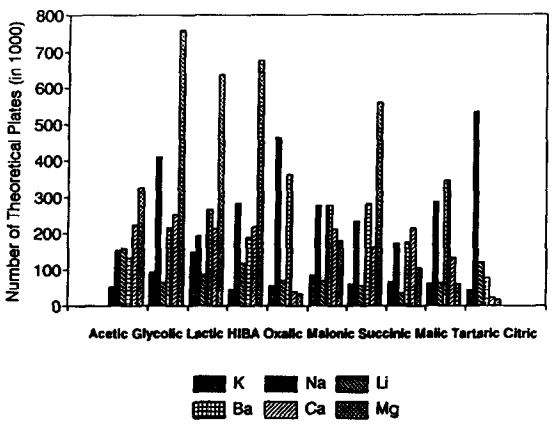

Fig. 8. Comparison of number of theoretical plates in different complexing agents. Conditions as in Fig. 7.

TABLE II

COMPARISON OF PRECISIONS WITH THE TWO INJECTION MODES

t = Migration time; A = peak area; H = peak height.

Ion	R.S.D. (%)[a]					
	EK mode			HD mode		
	t	A	H	t	A	H
K	0.73	5.8	3.5	0.80	6.5	3.6
Ba	0.83	3.3	2.7	0.63	5.5	1.8
Ca	1.02	3.8	2.8	0.59	4.8	2.2
Na	1.02	5.2	1.9	0.58	4.9	2.8
Mg	1.02	2.7	0.9	0.47	2.6	1.2
Li	1.30	1.2	1.0	0.69	2.2	1.3

[a] $n = 5$.

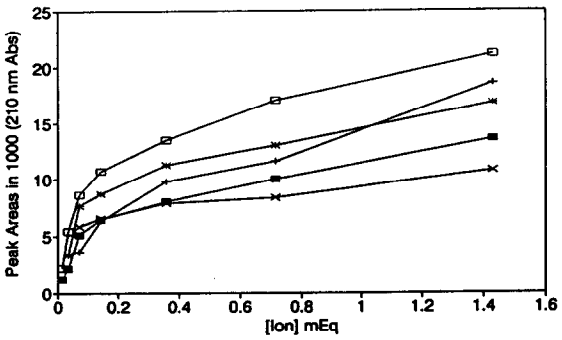

Fig. 9. Comparison of calibration graphs for the various ions in the presence of 3.0 m*M* succinic acid (pH 4.5). The pH was adjusted with 1 *M* succinic acid: × = Na; ■ = Li; + = Ba; * = Ca; □ = Mg.

included in this analysis) exhibit hyperbola-shaped curves. It is apparent that the calibration sensitivity is much better in the lower concentration range. Therefore, for quantitative analysis, the sample should be diluted serially such that the concentration of the analyte will fall into the range where the calibration graph is linear. The non-linearity problem in the indirect detection method has been noted previously [8]. Weston *et al.* [5] reported a good linearity in the low concentration range (2–160 ppb) over only 1.5 orders of magnitude. Beck and Engelhardt [8] reported that whereas a linear correlation with peak area exists for ion concentrations between 2 and 10 ppm, signal saturation with peak height occurs above 4 ppm. We found that the linearity and sensitivity are indeed better at the sub-ppm and tens of ppb level. The detection limit in this study is conservatively estimated to be at the tens of ppb to sub-ppm level and in good agreement with those reported by Weston *et al.* [5]. We are currently investigating methods for improving the precision, linearity and detection limit.

CONCLUSIONS

CE of alkali and alkaline earth metal ions using indirect UV detection with imidazole as background carrier buffer in conjunction with a complexing agent can be performed efficiently. When choosing a complexing agent, the pH of the carrier buffer should be around the pK_a of the complexing acid. If a diprotic acid is used, the pH must be between pK_1 and pK_2 value of the complexing acid. If sulphuric acid is used instead of HCl to adjust the pH, it will compete with the complexing agent and produce results different to those in its absence.

Monoprotic HIBA and lactic acid, offering a wider applicable pH range with good separation, migration time span and plate number, are good complexing agents for analyses for inorganic metal cations. Use of acetic acid is more limited (pH range 6.0–6.5) owing to its higher pK_a value, being closer to the operating range of imidazole buffer. The separation in glycolic acid is more sensitive to pH and the operating pH range is narrower than in HIBA or lactic acid (pH 3.5–5.5 *vs.* pH 3.5–6.0).

Diprotic oxalic, malonic and succinic acid, which provide a wider migration time span with a wider peak-to-peak separation time, are also good choices if manipulation of the migration times of divalent ions is desired. The migration time and order of Ba, Ca and Mg ions in these three acids are particularly sensitive to pH changes. Hydroxy diprotic malic and tartaric acid have a narrower operating pH range because the difference in pK_1 and pK_2 values is smaller than for the former two diprotic acids (1.3 *vs.* 3.0 pH unit difference). They are suitable for analysis at pH near 4.0.

Triprotic citric acid has a higher stability constant toward Group IIA metal ions. It provides a unique way to manipulate the mobility of divalent ions by varying the pH. Its drawbacks are a poorer plate number, slower migration time for the divalent ions and a limited pH operating range.

The migration order of monovalent ions, K < Na < Li, is always the same, independent of the type of complexing agent present. The migration order of divalent ions, Ba < Ca < Mg, is also the same and independent of the complexing acid used, but the migration times overlap with those of Na and Li. Hence the overall migration order and time span depend strongly on the running pH and the kind of complexing acid used. They are little affected by the monoprotic acids but are affects substantially by the complexing agent with the highest stability constant.

For routine determinations of these metal ions in real samples, we recommend CE running conditions as specified under Experimental. In addition, the following situations with regard to the complexing agent employed should be considered. (a) When the concentrations of all ions are comparable (and diluted to 0.1–1.0 mequiv.), using malonic acid (pH 4.0) or succinic acid (pH 4.5) will give the best separation. Using glycolic or lactic acid or HIBA at pH 4.0 will also provide good separation. (b) When the concentration of one ion species in the sample is particularly high (*e.g.*, in serum the Na concentration is approximately 40–50 times higher than those of K, Mg, or Ca), using oxalic acid (pH 4.0) or citric acid (pH 4.5) will avoid the problem of overlap of the large Na peak with the small peaks of the other three ions. As the ion concentration in real samples can vary greatly from sample to sample, the above conditions can only be regarded as a starting point and fine tuning is required for each individual circumstance.

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