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Prediction and optimization of the separation of metal cations by capillary electrophoresis with indirect UV detection

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ABSTRACT

The migration of metal cations in capillary electrophoresis with indirect UV detection was investigated. A mathematical model was used to relate the electrophoretic mobility of a metal cation with two background electrolyte factors: the pH and the concentration of a complexing agent. Imidazole, which has an electrophoretic mobility close to those of metal cations, was used as the visualization agent, and 2-hydroxyisobutyric acid (HIBA) was used as the complexing agent. Through computer modeling, the electrophoretic mobilities of fourteen metal cations were predicted on the basis of five initial experiments within the factor space of pH and HIBA concentration. Good agreement was observed between the predicted electrophoretic mobilities and the observed values of the metal cations. Subsequently, the separation of a complex mixture of metal cations was successfully optimized. A complete separation of 14 metal cations including alkali, alkaline earth and transition metal(II) cations was achieved within 4 min. In addition, the effectiveness of other visualization agents and complexing agents for the separation of metal cations was examined.

INTRODUCTION

Chromatographic methods for the separation of metal cations are time-consuming and often require sophisticated analytical columns and eluents [1,2]. Capillary electrophoresis (CE) is a separation technique undergoing rapid development in recent years. Its popularity has been largely attributed to high efficiency, short separation time, feasibility in selectivity manipulation and a relatively simple instrumental setup [3,4]. Separation in CE is based on the differential migration of ionic solutes. In principle, CE is well suited for direct analysis of inorganic ions such as metal cations. Sensitive detection of metal cations, however, is not feasible due to low molar absorptivities.

One solution to such a problem is to introduce chromophores or fluorophores in the buffer which form complexes with metal ions and can be detected spectroscopically. Examples such as laser fluorescence detection of metal-8-hydroxyquinoline-5-sulfonic acid complexes [5], or UV absorbance detection of cyanide complexes of iron(II) and iron(III) [6] have been reported.

Another approach is indirect photometric detection, which has been commonly used in ion chromatography for metal cation analysis [7,8]. To achieve indirect photometric or UV detection, an ion, called visualization agent which absorbs light in the UV–Vis region, is added to the background electrolyte in order to create a high background signal. The analyte ions are indirectly detected by the change in background signal when the analyte ions displace the visualization agent ions from the buffer according to charge conservation. Indirect fluorescence detec-

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tion is based on the same mechanism, except that a fluorophore is added to the background electrolyte.

The application of CE with indirect UV detection was first reported by Hjertén *et al.* [9] for the analysis of inorganic and organic anions. Indirect detection methods for CE have been recently reviewed by Yeung [10]. Current research is aimed at extending the number of applications of this technique, primarily by using different visualization and complexing agents to improve the separation. Foret *et al.* [11] reported the separations of metal cations by using 2-hydroxyisobutyric acid (HIBA) as a complexing agent and creatinine as a visualization agent on a polyacrylamide coated capillary. Weston and coworkers [12–14] reported the separations of metal cations using unspecified buffer conditions. The use of aromatic amines and heterocycle compounds as visualization agents have been reported by Chen and Cassidy [15] for lanthanide separation on bonded-phase capillaries, and by Beck and Engelhardt [16] for separations of alkali and alkaline earth and aliphatic amine cations.

In this paper, a simple mathematical model is used to relate the electrophoretic mobility of a metal cation to two experimental factors of the pH and the concentration of a complexing agent. These two factors have a pronounced effect on the complexation, and therefore, on the migration of metal cations. Consequently, electrophoretic mobilities of metal cations were predicted on the basis of a small number of experiments, and the separation of a complex mixture of metal cations was optimized. A complete separation of fourteen metal cations was achieved within 4 min. The effectiveness of different visualization and complexing agents on the metal cation separation is also examined.

EXPERIMENTAL

Apparatus

Experiments were carried out on a laboratory-built CE system. The system was composed of a 0–30 kV high-voltage power supply (Series EH; Glassman High Voltage, Whitehouse Station, NJ, USA), a variable-wavelength UV detector (Model 200; Linear Instruments, Reno, NV, USA) operated at 214 nm with 0.1 s rising time, and 50 μm I.D. × 375 μm O.D. fused-silica capillary tubing (Polymicro Technologies, Phoenix, AZ, USA). The total length of the capillary was 62 cm and detection was performed at 50 cm downstream. The capillary temperature was maintained at 30°C by jacketing it in light mineral oil using a constant temperature circulator (Type K2-R; Lauda, Germany). The samples were introduced from the anodic end of the capillary by gravity, 10 cm height for 5 s. Positive voltage of 25 kV was used for all the electrophoretic separations. Electropherograms were recorded with an integrator (Model SP4200; Spectra-Physics, San Jose, CA, USA).

Reagents and chemicals

The metal ion solutions were prepared by the dilution of 1000 μg/ml standard solutions of metal chlorides or nitrates obtained from Aldrich (Milwaukee, WI, USA). The UV visualization agents imidazole, benzylamine and trimethylbenzylamine hydroxide, and the complexing agents HIBA, citric acid and tartaric acid, are reagent grade and obtained from Aldrich. All other chemicals are of analytical-reagent grade.

Procedure

All background electrolytes contained 6 m*M* imidazole or other visualization agents. The concentration of the complexing agent, HIBA, varied from 2 to 20 m*M*. Solutions were prepared fresh daily using purified water (Milli-Q laboratory water-purification system, Millipore, Bedford, MA, USA). The pH values of the background electrolytes were adjusted using 1.0 *M* acetic acid or 0.5 *M* tetramethylammonium hydroxide. The typical concentration of metal cations in the sample mixture was about 10 μg/ml.

From a typical electropherogram, the electrophoretic mobility of an ionic solute (μ_{ep}) can be calculated from the mobility of the electroosmotic flow (μ_{eo}), and the observed mobility of the solute (μ_{ob}) according to:

$$\mu_{ep} = \mu_{ob} - \mu_{eo} = \left(\frac{1}{t_R} - \frac{1}{t_{eo}}\right) \cdot \left(\frac{L_t L_d}{V}\right) \qquad (1)$$

where L_t is the total length of capillary and L_d is the length of capillary to detector. V is the applied voltage. t_R and t_{eo} are the migration times of an analyte ion and neutral marker.

The velocity of the electroosmotic flow was estimated by injecting water. Migration times of the solutes were compiled and their electrophoretic mobilities were calculated using Quattro Pro spreadsheet (Borland International, Scotts Valley, CA, USA). Computer modeling, separation optimization and simulation were performed by using CAMOS (Computer Assisted Multivariate Optimization Strategies), a computer program developed in this laboratory in Turbo vision (Turbo Pascal Version 6.0, Borland International).

RESULTS AND DISCUSSION

In aqueous solutions of metal salts, solvated metal ions can exist as metal cations, or metal anions, or a series of hydroxymetal species depending on the oxidation state of the metals and the pH. For example, Fe(II) exists as cationic $Fe(H_2O)_6^{2+}$ in aqueous solution. Fe(III) shows a strong tendency to hydrolyze to form a series of hydroxyl species such as $Fe(OH)(H_2O)_5^{2+}$ and $Fe(OH)_2(H_2O)_4^{+}$ etc. Examples of metal anions include CrO_4^{2-} and VO_4^{3-}. In this report, since a positively charged visualization agent is used in the background electrolytes, this will limit the application of CE with indirect UV detection to metal cations which include alkali, alkaline earth and the transition metal(II) cations etc.

Imidazole as a visualization agent in CE with indirect UV detection was reportedly the most efficient in the separations of alkali and alkaline earth cations because its electrophoretic mobility is close to those of metal cations [16]. HIBA, a weak acid, has also been commonly used in the separation of metal ions including the lanthanides, alkali, alkaline earth and certain transition metal cations [11–15]. Since it is a weak complexing agent, metal ions are only partially complexed and are mostly in solution as cations. In this report, the use of quantitative description of migration of metal cations in the imidazole–HIBA system is examined.

Migration models for metal cations

The effect of pH and the concentration of a complexing agent (HL) on the electrophoretic mobility of a metal cation can be expressed by a phenomenological model; that is, the electrophoretic mobility of a metal cation equals the weighted sum of mobilities of free metal cation and various complexed species [5]:

$$\mu_{ep} = \alpha_M \mu_M + \alpha_{ML} \mu_{ML} + \alpha_{ML2} \mu_{ML2} + \cdots \qquad (2)$$

where μ_M, μ_{ML} and μ_{ML2} are the electrophoretic mobilities of various metal species; α is the molar fraction of each metal species. Therefore, similar to the treatment in ion chromatography [17], the electrophoretic mobility of a metal cation can be derived as:

$$\mu_{ep} = \frac{\mu_M + \mu_{ML}\beta_1\alpha_L C_{HL} + \mu_{ML2}\beta_2(\alpha_L C_{HL})^2 + \cdots}{1 + \beta_1\alpha_L C_{HL} + \beta_2(\alpha_L C_{HL})^2 + \cdots} \qquad (3)$$

where β_i ($i = 1, 2, \ldots n$) is the overall formation constant, n refers to the number of ligand involved, C_{HL} is the total concentration of complexing agent in the background electrolyte, α_L is the molar fraction of complexing agent capable of complexing with metal ion M, and is given by:

$$\alpha_L = \frac{K_a}{1 + K_a/[H^+]} \qquad (4)$$

where K_a is the acid dissociation constant of HL.

Substituting eqn. 4 into eqn. 3 would result in a very complex equation which directly shows the relationship between the electrophoretic mobility of a metal cation and the two important controllable factors (pH and C_{HL}). Swaile and Sepaniak [5] predicted the electrophoretic mobilities of Ca(II) and Mg(II) by simultaneously solving eqn. 3 on the basis of experimental data and literature values of the formation constants and the acid dissociation constant. A similar qualitative prediction of the retention of metal ions in ion chromatography was also reported by Karcher and Krull [17].

In this study, non-linear regression of electrophoretic mobility change as a function of pH and CL using eqn. 3 failed for two apparent reasons.

First, there are too many parameters (β, K_a and μ values) involved in the modeling process, and second, the number of parameters in eqn. 3 changes with the number of metal–ligand complexes. Instead, an empirical approach was chosen. The advantages of using an empirical approach include simplicity and generality. Consequently, fewer experiments are required and any combination of factors can be modeled by empirical approach. In addition, one general equation is applicable to all metal cations. One disadvantage of using an empirical model, however, is the loss of physically meaningful information about metal–ligand interactions.

A general linear model with interaction term was examined to relate the electrophoretic mobility and experimental factors (pH and C_{HL}). After a series of modeling tests, the following equation was found suitable in the range of the defined factor space:

$$\mu_{ep} = k_0 + k_1 C_{HL} + k_2 \cdot \mathrm{pH} \cdot C_{HL} + k_3 C_{HL}^2 \qquad (5)$$

where k_0, k_1, k_2, k_3 are empirical parameters.

Since eqn. 5 contains only four parameters, five experimental points were used in the model building step by using the least-square approach. The typical mobility responses for four metal cations as a function of the pH and HIBA concentration of background electrolyte are shown in Fig. 1. The electrophoretic mobilities decrease with increasing pH and/or HIBA concentration due to the greater complexing ability of the ligand. For alkali metal cations, no changes in electrophoretic mobility are observed due to the lack of complexation. The most dramatic changes are observed for the transition metal(II) cations such as Ni(II), Co(II), Cu(II) and Zn(II). The practical upper pH limit of approximately 5.0 was established because the protonation of imidazole (pK_a = 6.9) is incomplete and transition metal cations may hydrolyze at higher pH. The lower end of the operating pH was found to be approximately pH 3.5. At more acidic pH values, much of the complexing ability of HIBA is lost and the detection sensitivity of the metal cations is lower because of the higher concentration of H^+ in the background electrolyte.

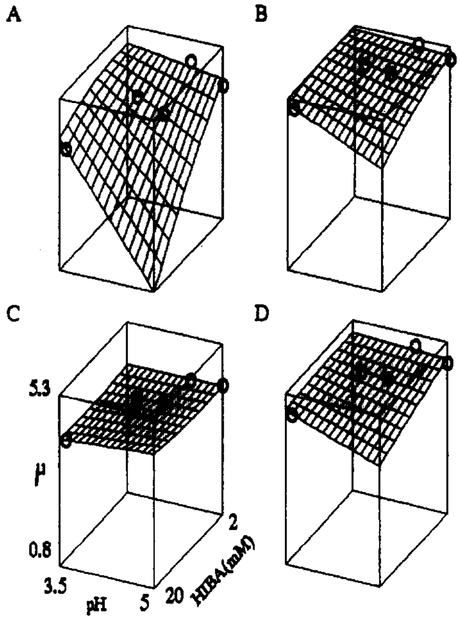

Fig. 1. Typical electrophoretic mobility responses of metal cations as a function of pH and HIBA concentration, predicted based on eqn. 5 and on five experiments shown in the figure. (A) Ni(II), (B) Mg^{2+}, (C) Li^+, (D) Cd(II). ($\mu \times 10^{-4}$ cm^2 V^{-1} s^{-1}).

In order to illustrate the prediction capabilities of the proposed model, the calculated electrophoretic mobilities of fourteen metal cations at thirteen different buffer conditions are plotted against the observed values as shown in Fig. 2 (n = 182). A correlation coefficient of 0.993 was obtained, indicating that the electrophoretic

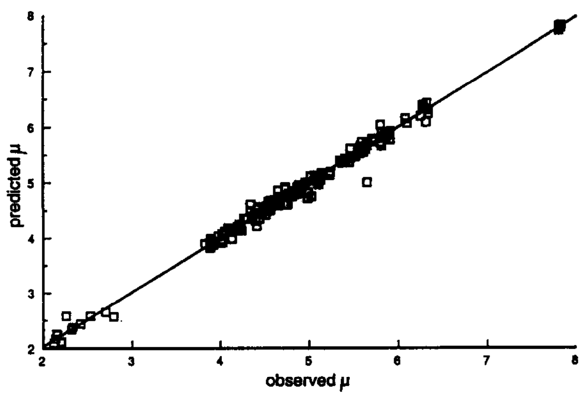

Fig. 2. The correlation plot of observed and calculated electrophoretic mobilities of fourteen metal cations at thirteen different experimental conditions ($\mu \times 10^{-4}$ cm^2 V^{-1} s^{-1}). The correlation coefficient (R^2) is 0.993.

mobility of a metal cation in imidazole–HIBA system can be accurately predicted on the basis of five experimental points. Consequently, the separation of a metal cation mixture can be optimized. The minimum resolution map for the separation of a mixture of fourteen metal cations is shown in Fig. 3. The resolution is calculated according to [3,4]:

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\mu_2 - \mu_1}{\mu_{avg} + \mu_{eo}}\right)$$

where N is the number of the theoretical plates. μ_1 and μ_2 are the electrophoretic mobilities of two neighboring metal cations, and μ_{avg} and μ_{eo} are their average and the electroosmotic mobility, respectively. As shown in Fig. 3, the optimum separation condition is in the region of the darker area pointed by an arrow. Fig. 4 shows the predicted effect of HIBA concentration on the electrophoretic mobilities and the minimum resolution plot for the separation of fourteen metal cations at pH 4.0. The minimum resolution plot shows that the optimum concentration of HIBA is about 12 m*M*. The corresponding separation is simulated and shown in Fig. 5A. The actual separation of these fourteen metal cations under the optimized condition is shown in Fig. 5B. The predicted separation (Fig. 5A) agrees very well with the actual one (Fig. 5B) in both elution order and migration times. Complete separation of fourteen metal cations including alkali and alkaline earth and transition metal(II) cations is achieved in less than 4 min. For the simulated electropherogram, t_0 or μ_{eo} as a system variable was estimated from the t_0 values of the same five experimental points. In addition, a system peak was observed after the elution of all metal cations, and before the large water peak (neither peaks are shown in the electropherogram). A similar system peak was also reported in the literature [18].

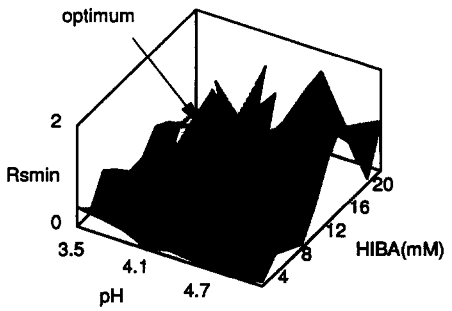

Fig. 3. Predicted minimum resolution map as a function of pH and HIBA concentration for a mixture of fourteen metal cations.

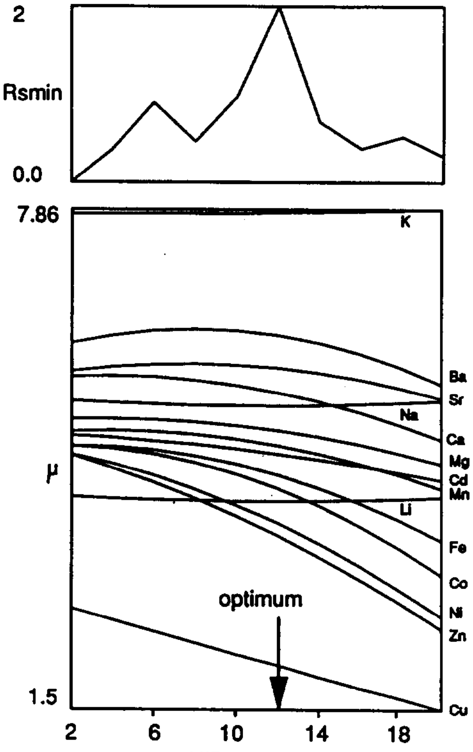

Fig. 4. Predicted effect of HIBA concentration on electrophoretic mobilities of fourteen metal cations and minimum resolution plot for a mixture of fourteen metal cations at pH 4.0 ($\mu \times 10^{-4}$ cm^2 V^{-1} s^{-1}).

The use of other visualization agents and complexing agents

As in ion chromatography, many water soluble aromatic amines and heterocycle compounds with high molar absorptivities have great potential for indirect UV detection of metal cations [7,8]. Fig. 6 shows the separation of the same metal cations as in Fig. 5, using benzylamine as the visualization agent. The electrophoretic mobility of benzylamine is less than imidazole in the buffer system. Higher efficiencies are ob-

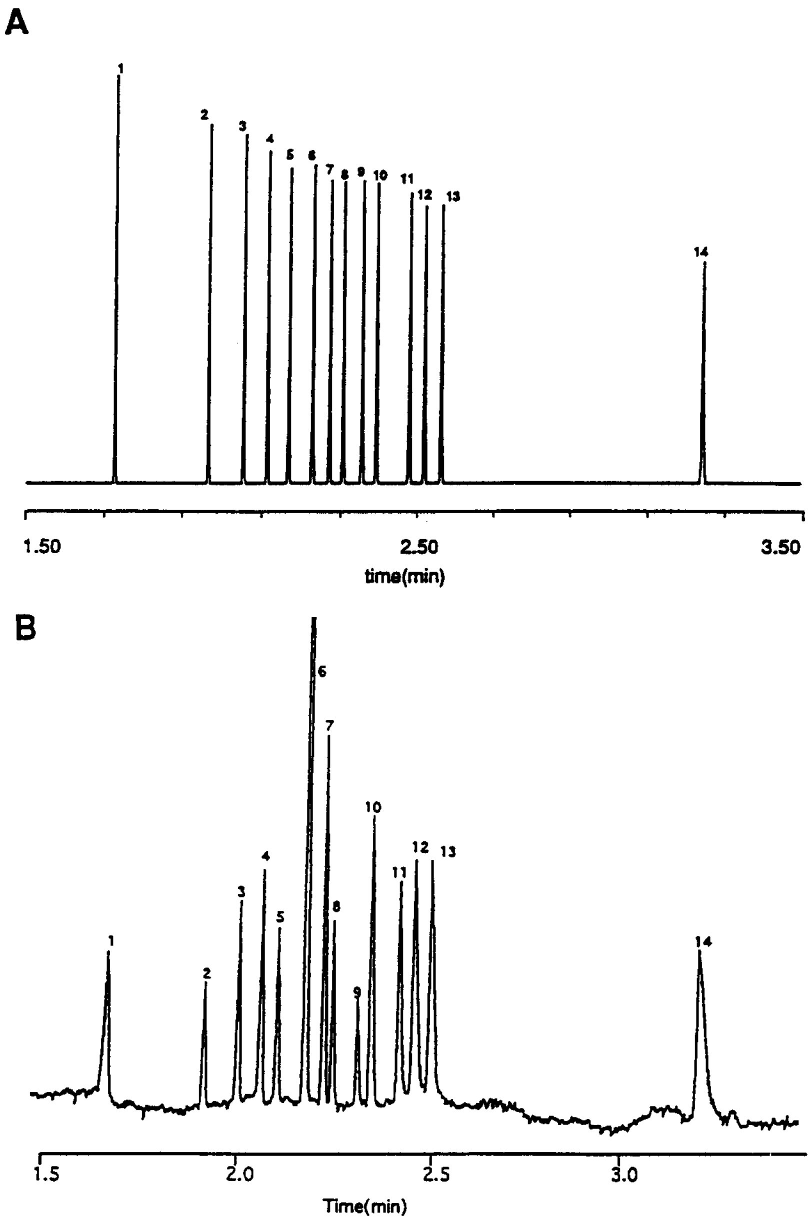

Fig. 5. Predicted separation at 12 m*M* HIBA, pH 4.0 (A) and the actual separation of fourteen metal cations (B). The background electrolyte: 6.0 m*M* imidazole, 12 m*M* HIBA, pH 3.95. Peaks: 1 = K^+; 2 = Ba^{2+}; 3 = Sr^{2+}; 4 = Ca^{2+}; 5 = Na^+; 6 = Mg^{2+}; 7 = Mn(II); 8 = Cd(II); 9 = Fe(II); 10 = Co(II); 11 = Li^+; 12 = Ni(II); 13 = Zn(II); 14 = Cu(II).

tained for late-eluting metal cations such as Ni(II), Zn(II) and Cu(II). Early-eluting metal cations such as alkali and alkaline earth, however, are less efficient due to the larger differences in electrophoretic mobility of metal cations and visualization agent. Nevertheless, the potential application of aromatic amines such as benzylamine and trimethylbenzylammonium cation is for the separation of slower migrating cations. In addition, these visualization agents remain as cations over a much wider pH range than imidazole (pK_a 6.9 for imidazole). There-

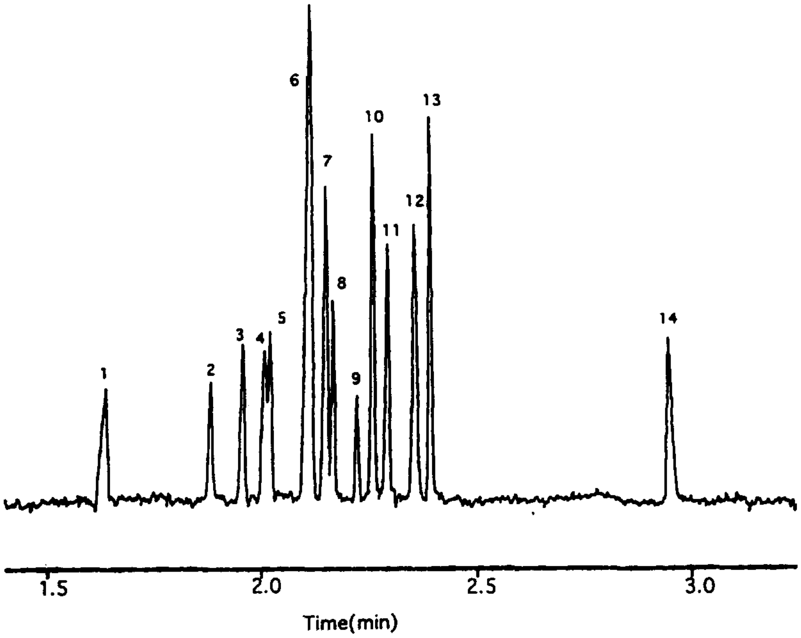

Fig. 6. Separation of the same mixture of metal cations as in Fig. 5. The background electrolyte: 6.0 m*M* benzylamine, 12 m*M* HIBA, pH 4.00.

fore, they are more suitable when higher pH values are required for the separation.

Like HIBA, other polycarboxylic acids can also be used in the background electrolyte to influence the electrophoretic mobility of metal cations, and to control selectivity. In general, the effectiveness of complexation increases in the sequence HIBA < tartaric acid < oxalic acid < citric acid [19]. For example, aqueous samples will often contain divalent metal cations (especially Ca^{2+} and Mg^{2+}) as well as alkali metal cations and ammonium ion. Fig. 7 shows separations of tap water using two different complexing agents. With the background electrolyte containing 4 m*M* tartaric acid, a stronger complexing agent than HIBA, Ca^{2+} and Mg^{2+} peaks are moved far behind the sodium peak (Fig. 7B) so that the separation of these two divalent cations from sodium are maximized without using very high concentration of HIBA and adding organic base to raise the pH of background electrolyte [14].

Another application of this technique is for the analysis of non-UV-absorbing organic amines as shown in Fig. 8. The amines are separated according to their molecular size and detected through displacing visualization cations in the background electrolyte.

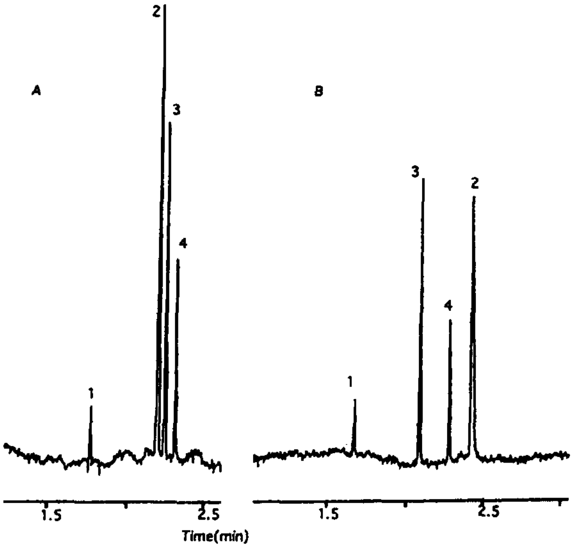

Fig. 7. Separations of tap water. Background electrolyte: 6 m*M* imidazole, (A) 12 m*M* HIBA, pH 3.95, or (B) 4 m*M* tartaric acid, pH 4.00. Peaks: 1 = K^{+}; 2 = Na^{+}; 3 = Ca^{2+}; 4 = Mg^{2+}.

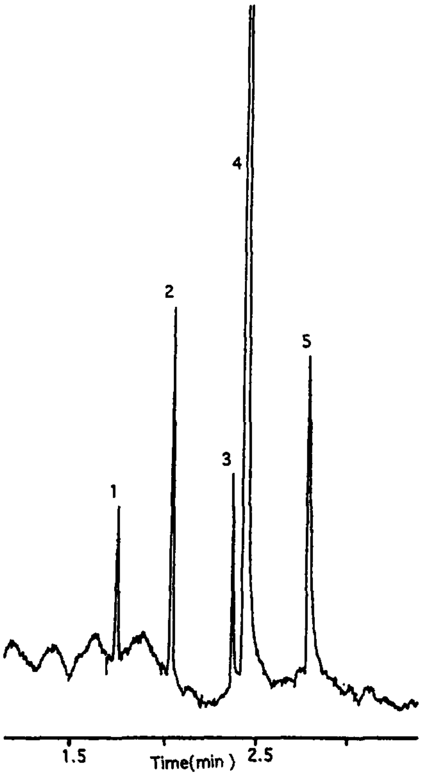

Fig. 8. Separation of aliphatic amines. Background electrolyte: 6 m*M* imidazole, 12 m*M* HIBA, pH 3.95. Peaks: 1 = NH_4^+; 2 = $CH_3NH_3^+$; 3 = $HO(CH_2)_2NH_3^+$; 4 = $(CH_3)_4N^+$; 5 = $(C_2H_5)_3NH^+$.

CONCLUSIONS

Using computer-assisted modeling and simulation, the electrophoretic mobility of metal cations can be successfully predicted on the basis of a small number of measurements. Subsequently, the separation of a complex mixture of metal cations can be easily optimized. As predicted, fourteen metal cations including alkali, alkaline earth and transition metal cations are completely separated in less than 4 min. Although the current method is applied to imidazole–HIBA system, the concept is entirely general and can be extended to other visualization–complexing agent system.

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