Effect of electrolyte composition on the separation of inorganic metal cations by capillary ion electrophoresis

Andrea Weston and Phyllis R. Brown*

Department of Chemistry, University of Rhode Island, Kingston, RI 02881 (USA)

Allan L. Heckenberg, Petr Jandik and William R. Jones

Ion Analysis Department, Waters Chromatography Division of Millipore, 34 Maple Street, Milford, MA 01757 (USA)

ABSTRACT

Capillary ion electrophoresis (CIE; Waters' tradename Capillary Ion Analysis) is a capillary electrophoresis technique in which the conditions for separation are optimized for the analysis of inorganic or low-molecular-weight ions. Among the variable parameters in CIE, control of the electrolyte composition provides one of the more significant means for manipulating separations. In the separation by CIE of mixtures of various alkali metal, alkaline earth metals and transition metals their relative selectivity could be changed by altering the pH and the concentration of the complexing agent in the electrolyte. To permit the use of indirect photometric detection and to ensure symmetrical peak shape a highly UV absorbing amine, with an electrophoretic mobility similar to those of the analyte cations, was chosen as the major component of the electrolyte. The reproducibility in terms of the migration times was less than 0.5% relative standard deviation and in terms of peak area was better than 2% relative standard deviation. Detection limits ranged from low to mid-parts per billion (10^9). Applications of this technique for the analysis of metal cations in tap water, orange juice and in acid-etching baths is demonstrated.

INTRODUCTION

Cation-exchange chromatography is commonly used to analyze for inorganic metal cations [1,2], and while methodology for these applications is relatively well-developed, additional improvements are still being made [3]. Although a fairly large number of reports have appeared in recent years describing separations of inorganic metal cations by isotachophoresis [4–10], only a few applications by capillary electrophoresis (CE) have been reported [11–16]. Capillary ion electrophoresis (CIE; Waters' tradename Capillary Ion Analysis) is a CE technique in which the conditions for separation are optimized for the analysis of inorganic or low-molecular-weight ions.

There are a number of variables, such as the hardware configuration and the electrolyte composition, which will affect the separation of analytes in CIE. While the mode of detection, the applied voltage and even the length of the capillary may affect the separation, the focus of this paper is the effects of the electrolyte composition on the separation.

Separations by CIE are based on differences in the electrophoretic mobilities of the injected ions [17, 18]. Electrophoretic mobilities are influenced by the structural properties of solutes such as size, shape, Stokes' radius, charge and mass, in addition to the interaction of those solutes with the carrier electrolyte. Each of these variables is affected by properties of the electrolyte such as pH, ionic strength or viscosity. When employing indirect photometric detection, the chemical nature and the concentration of the UV active component are also factors which influence the separation [19].

Reports in recent literature have described methods to improve peak resolution by manipulating the mobilities of the biological analytes through variation of the electrolyte parameters [19,20]. Similar

principles were applied by Swaile and Sepaniak [21] to the determination of three metal ions, Ca(II), Mg(II) and Zn(II) by capillary electrophoresis, with on-column chelation using 8-hydroxyquinoline-5-sulfonic acid and indirect fluorescent detection.

In this paper, the effect of changing both the pH of the electrolyte and the nature and concentration of the complexing agent in the electrolyte on the separation and detection of a group of alkali, alkaline earth and transition metals by indirect UV detection will be discussed. The applicability of this means of modification of peak selectivity will be demonstrated by the separation of cations in various complex sample matrices.

EXPERIMENTAL

A Waters (Milford, MA, USA) Quanta 4000 capillary electrophoresis system, equipped with a positive power supply, was used throughout this study. Fused-silica capillaries, 60 cm total length, 75 μm internal diameter and 52 cm from the point of sample introduction to the detector window, were obtained from Waters (AccuSep capillaries). Indirect UV detection was achieved at 214 nm with a zinc lamp and a 214-nm optical filter, or at 185 nm with a mercury lamp and a 185-nm optical filter. The samples were introduced into the capillary using 30-s hydrostatic injections, from a height of 10 cm. The separation voltage was set at 20.00 kV.

Standard 2-ml polyethylene sample vials (Sun Brokers, Wilmington, NC, USA) were used as containers for the carrier electrolyte and all the standards and samples. A Waters 860 data station and Waters SIM interface were used to record and evaluate the electropherograms at a sample rate of 20 points per second. The subsequent statistical processing was performed using CricketGraph (Cricket Software, Malvern, PA, USA) with a Macintosh SE personal computer (Apple Computers, Cupertino, CA, USA).

All solutions, electrolytes and standards were prepared using 18-MΩ water generated by Milli-Q laboratory water purification system (Millipore, Bedford, MA, USA). The transition metal standards were prepared by the dilution of standards obtained from Sigma (St. Louis, MO, USA), while the alkali and alkaline earth metal standards were prepared from salts obtained from Aldrich (Mil-

waukee, WI, USA), as was the analytical reagent grade α-hydroxyisobutyric acid (HIBA). The UV background-providing component of the electrolyte, UVCat-1, was obtained from Waters. The concentration of UVCat-1 was held constant at 5 mM, while the HIBA concentration was varied as specified in the figures.

RESULTS AND DISCUSSION

The equivalent ionic conductivities [22] of the cations, λ_i, are directly related to the electrophoretic mobilities [11]. Theoretical selectivity of cation separations in CIE can be predicted on the basis of the value for each cation. The closer the equivalent ionic conductivities for each cation, the more challenging the separation. The equivalent ionic conductivities of the alkali metals are sufficiently different for their separation to be readily achieved [11]. For the remainder of the metals, however, differences in individual ionic mobilities alone do not provide a separation. To separate cations with similar ionic mobilities, an additional separation mechanism must be introduced. One frequently used mechanism is based on weak complexation [12], where the mobility of each cation is modulated by its degree of complexation with the complexing agent. Since each metal has a unique affinity for the complexing agent, their mobilities are altered to different extents, providing the differences in mobility needed for separation.

The velocity of the electroosmotic flow (EOF), and the electrophoretic mobilities together provide the "apparent" mobility which is measured in CIE

TABLE I

FORMATION CONSTANTS SHOWING THE DIFFERENCE IN AFFINITY FOR VARIOUS COMPLEXING AGENTS WITH DIFFERENT METAL CATIONS

Cation	Formation constant (log K)		
	Citrate	Oxalate	HIBA
Barium	2.98	2.31	0.36
Magnesium	3.29	3.43	0.81
Calcium	4.68	3.00	0.92
Manganese	3.67	3.97	0.96
Zinc	4.71	4.89	1.71
Copper	4.35	6.16	2.74

[11]. The "apparent" mobility may be reduced by use of a suitable complexation equilibrium, due to the reduction in the effective charge on the complexed metal. Table I shows the formation constants for three common complexing agents [23], with a variety of metals ions. The larger the formation constant, K, the lower the apparent charge on the cation and thus the slower the mobility of the cation would be expected to be. It is apparent from the table, that different complexing agents will provide different degrees of complexation with any given metal ion and that the choice of complexing agent will depend on the application. However, if too strong a complexing agent is used, the advantage of speed, provided by CIE, is compromised. The complexing agent used throughout this work was HIBA, due to its solubility and transparency in the UV-absorbing electrolyte, UVCat-1. It is stable
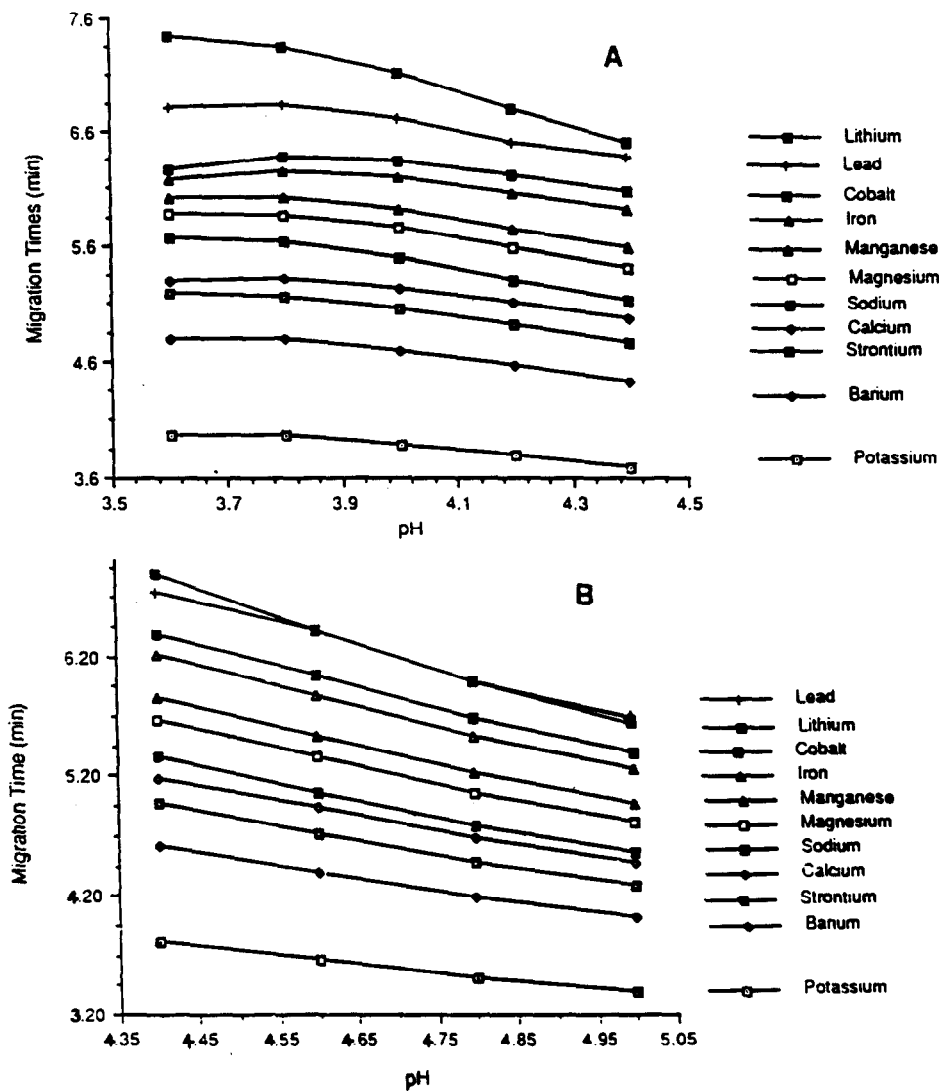

Fig. 1. (A) Graph showing the effect of decreasing the pH on the migration times of various inorganic metal cations. The electrolyte was 5 mM UVCat-1 with 6.5 mM HIBA, the natural pH of which was 4.4, and the pH was decreased using acetic acid. (B) Graph showing the effect of increasing the pH on the migration times of various inorganic metal cations. The electrolyte was the same as described in (A), except that the pH was raised using N,N-diethylaminoethanol.

under normal conditions and rapidly establishes a complexation equilibrium in the pH range of the electrolyte.

The interaction between the metal ion (M) and the complexing agent, as explained by Swaile and Sepaniak [21], can be described by the equilibrium expression

$$K' = \frac{[M(HIBA^-)_n]}{[M][HIBA^-]^n} \qquad (1)$$

where K' is the overall conditional formation constant for that metal ion and n is the number of ligands. The reaction occurs in a stepwise fashion with different metal–HIBA$^-$ complexes existing simultaneously. The observed electrophoretic mobility of the metal ion, μ_{obs}, can therefore be assumed to be a combination of the mobilities of the various forms of the metal present. The net mobility of the resulting analyte band, then, is determined by the distribution between the various possible forms of the metal complex.

The effect of the carrier electrolyte pH on the overall conditional formation constant is described by

$$K' = \alpha^n K_f \qquad (2)$$

where K_f is the conditional formation constant at

infinite dilution and α is the degree of protonation of the complexing agent. Reducing the pH, then, decreases the value of α and the concentration of the complex.

The effect of pH on the migration times of eleven alkali, alkaline earth and transition metals of altering the pH can be seen in Fig. 1. Using 6.5 mM HIBA, the natural pH of the electrolyte was pH 4.4. The pH was taken below pH 4.4 with acetic acid (Fig. 1A) and above pH 4.4 with N,N-diethylaminoethanol (Fig. 1B). There are two effects worth noting. Firstly, as the pH is lowered, the migration times increase, due to the decrease in the EOF. Likewise, as the pH is increased, the migration times decrease, due to the increase in the EOF. The velocity of the EOF is primarily a function of the number of dissociated, negatively charged silanol groups on the inside of the capillary wall. The more negatively charged silanol groups that are available, the greater the velocity of the EOF. As the pH is decreased, the number of dissociated silanol groups decreases and thus the velocity of the EOF also decreases.

The second point of interest, is that in addition to the minor change in mobility due to the EOF, the pH is also affecting the complexing equilibrium to a small extent. This becomes apparent by comparing
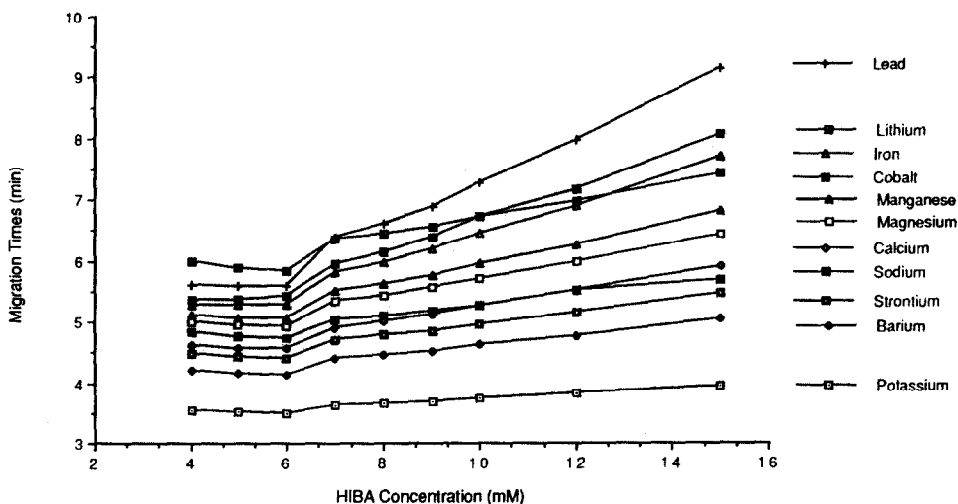

Fig. 2. Graph showing the effect of changing the concentration of HIBA on the migration times of a group of alkali, alkaline earth and transition metals. By altering the concentration of HIBA, the relative migration times of the metals can be changed. Below 6.5 mM HIBA, the pH was maintained at 4.4 with acetic acid. Above 6.5 mM HIBA, the pH was maintained at 4.4 with N,N-diethylaminoethanol. The background electrolyte was 5 mM UVCat-1.

the slopes of the migration times. The alkali metals do not form complexes and therefore their slopes can be expected to accurately reflect the change in mobility of the EOF with pH. However, the slopes of the other metals do not follow those of the alkali metals, indicating a secondary effect, namely the pH effect on the complexation equilibrium.

An even more significant effect on the selectivities of the cations can be seen by altering the concentration of HIBA, whilst maintaining the pH at 4.4. Fig. 2 shows the effect of changing the HIBA concentration on the migration times of the alkali, the alkaline earth and the transition metals. In order to maintain the pH at 4.4, below 6.5 mM acetic acid was used, while above pH 4.4 N,N-diethylaminoethanol was added. Since the alkali metals show no significant complexation with HIBA, and since their relative migration times do not change, the increase in migration times as the HIBA concentration is increased most likely reflects the decrease in the EOF due to a decrease in ionic strength with increasing HIBA. The relative migration times of the alkaline earth metals do change a little as the HIBA concentration increases, indicating that in addition to the effect of the EOF, the change in migration times is also due in part to increased complexation in the presence of additional HIBA. The effect of an increased amount of HIBA is even more pronounced with the transition metals where there is a significant increase in resolution between the metals at 15 mM HIBA when compared with 6 mM HIBA. By plotting all the data on a single graph, it becomes apparent that the elution order of the metals can be manipulated by altering the concentration of the complexing agent. The separation of 15 cations, including 8 transition metals, in less than 8 min using 6.5 mM HIBA as the complexing agent has been shown previously [11].

The minimum detectable concentrations for the metals, based on peak height of twice the baseline noise, range from low- to mid-ppb (10^9) levels [11] for the electrophoretic buffer containing UVCat-1 and HIBA at pH 4.4. The relative standard deviation (R.S.D.) in migration time for each of the group IA and group IIA cations, shown in Table II, was less than 0.5% ($n = 5$) while the R.S.D in peak area was less than 2%. The linear dynamic range was almost two orders of magnitude above the detection limit, the correlation coefficients ranging from 0.990

TABLE II

DATA FROM FIVE CONSECUTIVE INJECTIONS SHOWING THE REPRODUCIBILITY OF CIE IN MIGRATION TIMES AND PEAK AREA FOR A VARIETY OF ALKALI AND ALKALINE EARTH METALS

The electrolyte was 5 mM UVCat-1 with 6.5 mM HIBA, natural pH. All other conditions as in Fig. 3.

Cation	Migration time, R.S.D. (%)	Peak area, R.S.D. (%)
Lithium	0.38	1.40
Potassium	0.34	1.80
Sodium	0.37	1.22
Calcium	0.37	1.20
Magnesium	0.83	0.80
Barium	0.37	0.97
Strontium	0.38	1.07

for sodium to 1.000 for magnesium. The upper limit of calibration reflected a loss of resolution between the peaks rather than loss of linearity.

The potential of the discussed method for manipulating peak selectivity during the analysis of cations in complex matrices is shown in Figs. 3–5. In Fig. 3, which shows the relatively simple sample matrix tap water, the electrolyte employed to effect the separation was 5 mM UVCat-1 with 6.5 mM HIBA at pH 4.4, using indirect UV detection at 214 nm. The notable feature of this separation is that in addition to the alkali and alkaline earth cations expected in the sample, copper was also detectable in the same 8-min run.

A more complex, acidic matrix is shown in Fig. 4, where a sample of commercial orange juice was diluted 1:100 in Milli-Q water and injected without filtration. When analyzing for small quantities of metal ions, it is often undesirable to filter the sample, since cation contaminants may be introduced by the filters themselves. The fact that samples such as orange juice may be run without filtration may be of use for other samples, such as blood sera. The electrolyte for this separation contained 5 mM UVCcat-1, with 8 mM HIBA instead of 6.5 mM HIBA. The HIBA concentration was increased to improve the resolution between calcium and sodium. The indirect UV detection for this sample was carried out at 185 nm instead of at 214 nm. The
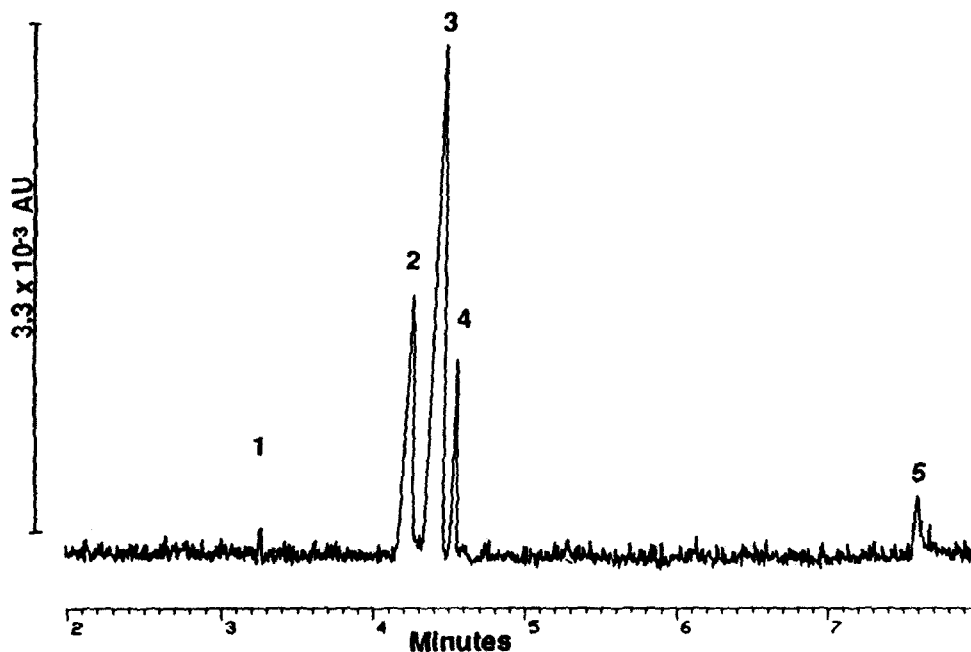
Fig. 3. CIE analysis of a tap water sample. Carrier electrolyte: 5 mM UVCat-1, 6.5 mM HIBA, pH 4.4; capillary: 60 cm × 75 μm I.D. fused silica; voltage: 20 kV (positive); hydrostatic injection: 30 s from 10 cm height; indirect UV detection at 214 nm. The sample was diluted 1:4 in Milli-Q water before injection. Peaks: 1 = potassium (0.3 ppm); 2 = calcium (4.3 ppm); 3 = sodium (12.5 ppm); 4 = magnesium (1.0 ppm); 5 = copper (1.1 ppm).
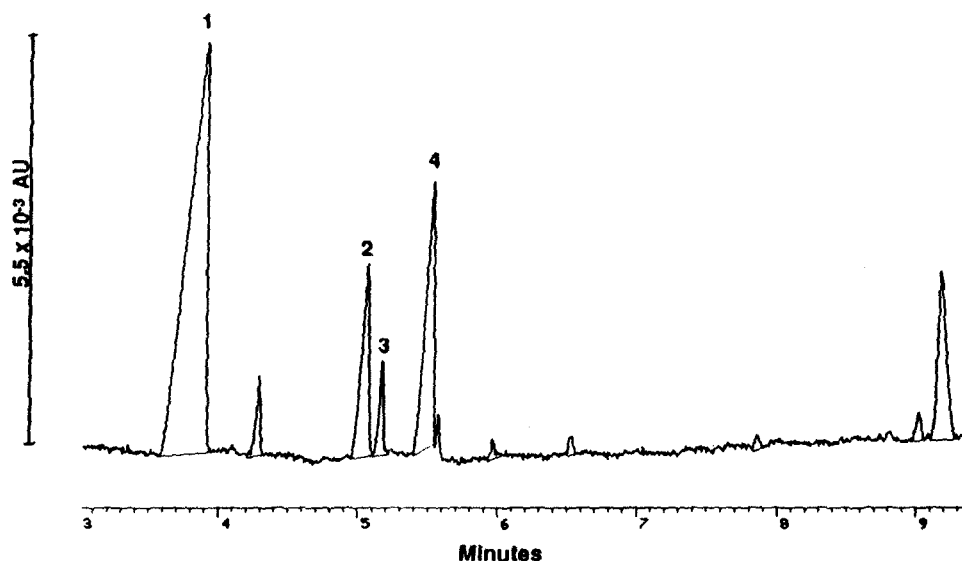

Fig. 4. CIE analysis of a commercial orange juice. Conditions as in Fig. 3 except the concentration of the complexing agent, HIBA is 8 mM and the detection system was indirect UV at 185 nm. The sample was diluted 1:100 in Milli-Q water before injection. Peaks: 1 = potassium; 2 = calcium; 3 = sodium; 4 = magnesium. Other peaks are inidentified.
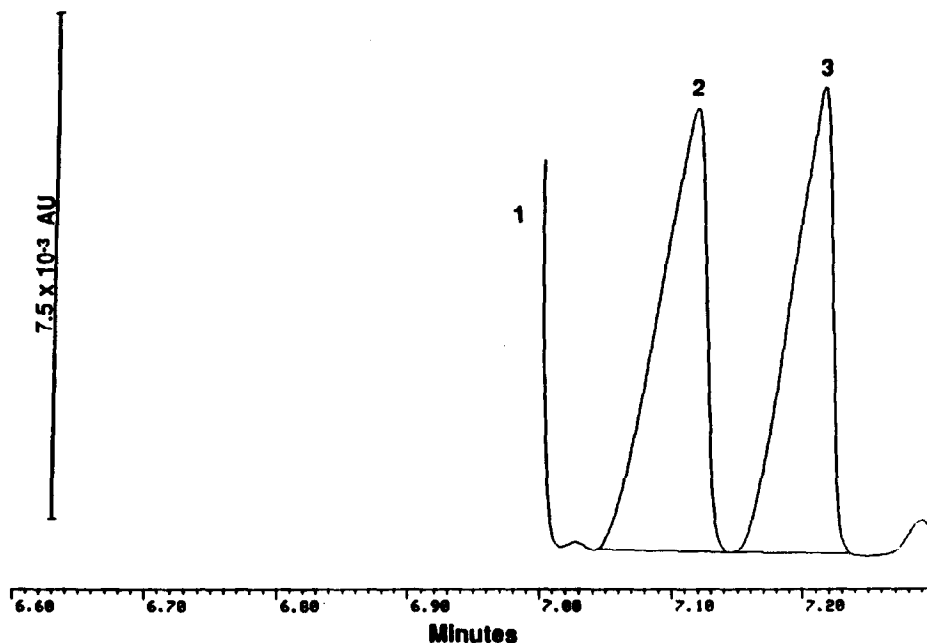
Fig. 5. CIE analysis of trace metals in an acid etching bath. Conditions are as described in Fig. 4. The sample was diluted 1:50 in Milli-Q water before injection. Peaks: 1 = sodium (1000 ppm); 2 = nickel (5 ppm); 3 = zinc (5 ppm).

change in detection wavelength was found to improve the sensitivity by a factor of 2.

The sample depicted in figure 5 was from an acid etching bath containing 5% phosphoric acid, 5% nitric acid and 5% sodium with trace amounts of nickel and zinc. The sample was diluted 1:50 in Milli-Q water, producing a solution containing 1000 ppm sodium. Nickel and zinc were added to the diluted sample to a concentration of 5 ppm and the separation effected with good baseline resolution, using 8 mM HIBA to enhance the resolution of the nickel and zinc from the sodium with indirect UV detection at 185 nm. Calculated detection limits for nickel and zinc in the original etching bath correspond to less than 5 ppm.

Electrophoretic buffer parameters which influence the complexation reaction also affect the detectability and efficiency in CE [20]. As the concentration of HIBA is increased, the metal–HIBA equilibrium is shifted towards the formation of complex, resulting in longer analysis times. However, if high HIBA concentrations are used to control the selectivity, large electrophoretic currents are observed which can degrade efficiency and which will affect detectability. In addition, in order to produce sharp peaks, the mobilities of the analyte ions should closely match the mobility of the corresponding ions in the electrolyte. As the concentration of HIBA is increased, the match of the analyte ion mobilities with the electrolyte mobility deteriorates due to the decreased ion mobilities. As a consequence, the efficiency of the separation decreases.

CIE offers the possibility for the rapid, reproducible and sensitive analysis of inorganic ions. With this method, peak selectivity can be manipulate and very low concentrations of cations can be determined in complex matrices.

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