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Separation and indirect detection by capillary zone electrophoresis of ppb (w/w) levels of aluminum ions in solutions of multiple cations

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Abstract

Solutions of multiple cations in aqueous solutions at concentrations as low as 200 ppb were analyzed by capillary zone electrophoresis. Aluminum ions were cleanly separated from Li^+, K^+, Ca^{2+}, Cr^{3+}, Zn^{2+}, Cu^{2+}, and other ions less than 6 min after injection of the solution on a 50 cm × 50 μm I.D. uncoated fused-silica capillary column at 15 kV. Indirect detection at 204 nm was carried out using a pH 2.8 background electrolyte containing 5.2 m*M* ephedrine as a UV-absorbing co-ion and 4.7 m*M* α-hydroxyisobutyric acid as a complexing counter ion. Mobilities for Al^{3+} and 14 other complexed cations were determined for this electrolyte.

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1. Introduction

Aluminum, an abundant element in the environment, presents the possibility of toxic effects on humans, plants and aquatic organisms. For example, Al toxicity may become a problem when plants are growing in acid soils. The solubility of Al and aqueous equilibrium behavior of Al species have been extensively studied, and merits of numerous techniques for analysis of Al have been recently reviewed [1]. However, there is a continuing need to develop new methods for the detection of soluble Al species, and we have sought to determine the conditions under which capillary zone electrophoresis (CZE) could be applied to the analysis of aqueous aluminum ions, since this technique offers certain advantages such as very small total sample size requirement (< 100 μl) and rapid separation of ions in aqueous solutions of multiple ions.

Because of a relationship suggested between Al and Alzheimer's disease and concerns about the possibility of ingesting Al in bioavailable forms from drinking water, Wu *et al.* [2] recently used CZE with indirect detection to study the distribution of fluoroaluminum species in water. Indirect detection relies on the displacement of light-absorbing species from the background "carrier" electrolyte by the analyte ions, and results in an electropherogram exhibiting series of negative peaks as the ions migrate past the optical window.

CZE has been applied to the analysis of numerous metal cations other than Al. Motomizu *et al.* [3] used a UV-absorbing chelate, ethylenediaminetetraacetic acid (EDTA), to detect complexes with alkaline earth metal ions directly in a pH 9.2 carrier solution. Multicom-

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ponent mixtures of transition metal complexes with 8-hydroxyquinoline-5-sulfonic acid were detected directly in borate buffer at pH 9.2 by Timerbaev *et al.* [4]. However, because of the unique solubility properties of Al compounds, it is usually desirable to carry out analyses for Al^{3+} at low pH. Foret *et al.* [5] studied rare earth ions in addition to Li, Na, K and Mg, and carried out analyses using indirect detection at 220 nm with a pH 4.8 electrolyte of 30 m*M* creatinine acetate and 4 m*M* α-hydroxyisobutyric acid (HIBA). The creatinine acetate acts as the UV-absorbing co-ion and the HIBA as a complexing counter ion.

Weston *et al.* [6,7] used Waters' UVCat 1 as the UV-absorbing co-ion together with HIBA as a complexing agent to study factors affecting separation of group IA, group IIA, transition metal and lanthanide cation mixtures. Indirect UV detection at 214 nm was used. They showed that cations with the most symmetrical peak shapes were those with electrophoretic mobilities close to that of the UV-absorbing component of the electrolyte. They also investigated the influence of changes in pH of the electrolyte. Lowering pH increased migration times due to increasing electroendosmotic flow that results from a change in the number of dissociated, negatively charged silanol groups on the inside of the capillary wall. A small effect of pH change on the complexing equilibrium was also noted.

Our studies to develop a CZE analysis method for Al began using imidazole as the UV-absorbing component of the electrolyte following the work of Wu *et al.* [2]. However, in our electrolyte containing HIBA as a complexing agent at pH 2.8, the peak for aluminum, which was one of the last to emerge from the column, was somewhat distorted. Using the mobility matching concept of Weston *et al.* [6,7] and the work of Beck and Engelhardt [8], who reported mobility data for several alternative UV-absorbing compounds, we selected ephedrine as our UV-absorbing electrolyte because its mobility more closely matched that of the Al ion and better peak shapes resulted.

2. Experimental

Solutions were analyzed using a BioFocus 3000 capillary electrophoresis system (Bio-Rad, Hercules, CA, USA). Fig. 1 shows a schematic diagram of the instrument. It features two rotating vial holders from which 0.5-ml sample vials are lifted and sealed gas-tight to the inlet and outlet of the column. An aliquot from the inlet vial may be injected by applying gas pressure to the vial to force sample liquid onto the capillary column or by turning on the high voltage for a period of time to cause ions to migrate into the column while the sample vial is in position at the column inlet. A normal operating sequence consists of (1) purging the column with electrolyte which is discharged into a waste vial, (2) rotating both carousels so that the sample to be injected is at the column inlet and the run electrolyte is at the exit, (3) injecting the sample, (4) rotating the inlet carousel to place run electrolyte at the column inlet and (5) turning on

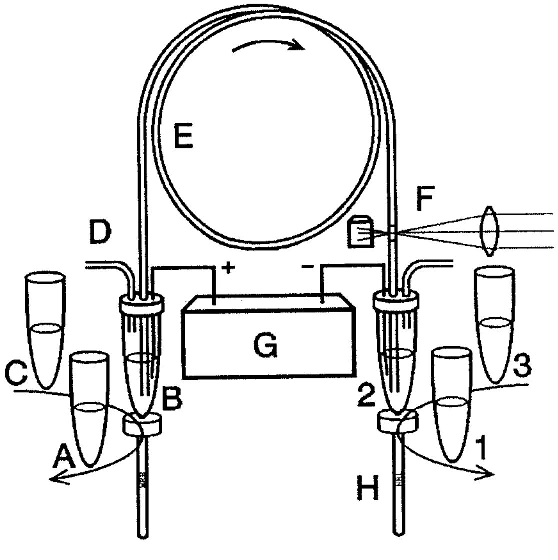

Fig. 1. CZE instrument shown in pressure injection mode. A = Purge solution; B = sample; C = inlet run electrolyte; D = helium inlet and gas-tight cap; E = capillary column; F = spectrometer beam through optical window; G = high-voltage source; H = vial lifter; 1, 3 = waste vials; 2 = outlet run electrolyte.

the voltage to make the run. Since only enough solution is required in the 0.5-ml sample vial to contact the column end for pressure injection, very small samples can be analyzed.

For this work the column was purged for 2 min three times with the background electrolyte before each sample injection. Samples were introduced onto the column from 0.5-ml polypropylene vials by pressure injection at 5 p.s.i. s (1 p.s.i. = 6894.76 Pa) or by electro-injection at 10 kV for 10 s. Runs were made at 15 kV for 10 min except where otherwise noted. The uncoated capillary column was cleaned at the end of each day by purging with triply distilled water and then drying with helium. The instrument was fitted with a cartridge containing a 50 cm × 50 μm I.D. uncoated capillary column. The background electrolyte containing 5.2 m*M* ephedrine and 4.7 m*M* HIBA (both from Sigma, St. Louis, MO, USA) was adjusted to pH 2.8 with Ultrex II HCl (J.T. Baker, Phillipsburg, NJ, USA). An optimum wavelength of 204 nm was chosen for indirect detection of the ions based on the UV spectrum of the ephedrine in the background electrolyte (Fig. 2). Test solutions were made by dilution of 1000 ppm atomic absorption metal reference standards (Sigma) with triply distilled water from an all quartz still. The distilled water was adjusted to $\text{pH} < 3$ with Ultrex II HCl before addition of the aliquot from the atomic absorption standard in order to maintain a low pH at all stages of sample handling.

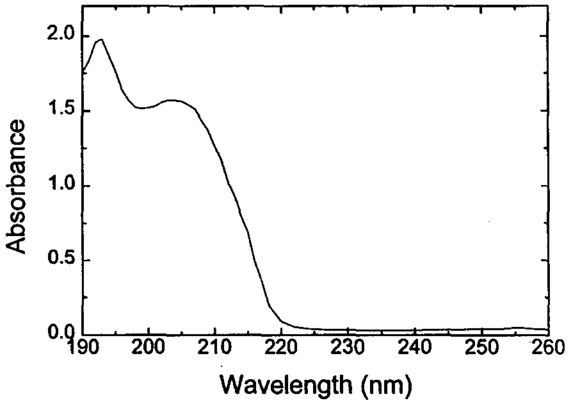

Fig. 2. UV spectrum of a solution of 5.23 m*M* ephedrine and 4.74 m*M* HIBA adjusted to pH 2.8 then diluted 1:25 and run in a 1-cm optical cell.

Since identification of unknowns by the indirect method is based primarily on migration times, a series of 15 solutions containing 10 ppm each of Na^+ and a second ion were injected to determine the relative order of migration times of the ions. The Na^+ was included as a check on the consistency of retention times from run to run.

3. Results

Mean migration times at 15 kV for Al and 15 other ions were determined by combining data for runs of the 10-ppm two-ion solutions with data for solutions of multiple ions at 200 ppb and 170 ppb. Mobilities of the ions interacting with the HIBA complexing agent were computed from the migration times, *t*, column length to the optical window, *L*, and separation voltage, *V* [6]:

$$\mu_m = L^2/Vt \tag{1}$$

These values are compared in Table 1 to mobilities of the free ions computed from published values of limiting equivalent ionic conductance at infinite dilution, λ, at 25°C [10]:

$$\mu_c = \lambda/F \tag{2}$$

where *F* is the Faraday constant, 96 500 C/equiv. and the appropriate unit conversions are made. Significant deviations between μ_m and μ_c are likely due to different degrees of ion–HIBA complex formation in the run electrolyte. Equilibria between HIBA and cations have been studied by Weston *et al.* [7], who also pointed out that the observed mobility of a single ion results from a combination of the mobilities of the various forms of the metal present. They found that formation constants (log *K*) of HIBA complexes with Ba, Mg, Ca, Zn and Cu varied from 0.36 for Ba to 2.74 for Cu while the p*K* of HIBA ($\text{HIBA}^0 = \text{H}^+ + \text{HIBA}^-$) is 3.717 [10]. This indicates that at low metal ion concentrations and at $\text{pH} < 3$ HIBA^0 is favored over the

Table 1
Mobility of metal–HIBA complexes in 5.2 m*M* ephedrine and 4.7 m*M* HIBA at pH 2.8

Ion	Mean migration time (min)[a]	Measured mobility, μ of complex ($cm^2\ kV^{-1}\ s^{-1}$)[b]	Calculated mobility, μ of free metal ion ($cm^2\ kV^{-1}\ s^{-1}$)[c]
Cd	5.57 ± 0.15	0.397	0.556
Cu	5.45 ± 0.14	0.406	0.570
Mg	5.08	0.435	0.550
Ni	5.07 ± 0.02	0.436	0.518
Co	5.02	0.440	0.549
Mn	5.02	0.440	0.554
Na	4.93 ± 0.06	0.448	0.519
Al	4.92 ± 0.06	0.449	0.632
Zn	4.88 ± 0.14	0.453	0.547
Cr	4.71 ± 0.08	0.469	0.694
Ca	4.43 ± 0.08	0.499	0.617
Sr	4.43	0.499	0.616
Ce	4.39	0.503	0.725
Ti	3.53 ± 0.04	0.626	0.788
K	3.47 ± 0.04	0.637	0.762
Li	3.21 ± 0.03	0.689	0.401

[a] Migration distance: 44.6 cm in a 50 μm I.D. uncoated capillary. Injection: 5 p.s.i. s. Run conditions: 15 kV; indirect detection at 204 nm. Cu, Al, Zn, Cr, Ca and K four runs at 10 ppm, 170 ppb, 200 ppb and 200 ppb. Cd, Ni, Na and Ti two runs at 10 ppm and 170 ppb. Li two runs both at 200 ppb. Mg, Co, Mn, Sr and Ce one run at 10 ppm.

[b] Computed from $\mu = L^2V^{-1}t^{-1}$ where $L = 44.6$ cm, $V = 15$ kV and t = mean migration time in column 2.

[c] Literature values of limiting equivalent ionic conductance at 25°C divided by the Faraday constant, 96 500 C/equiv. [10].

metal ion complexes. Also, the conditions within the column are more complicated than a simple equilibrium. Since various ions, complexes, and components of the electrolyte are moving through the column at different rates, the composition of the solution at any point within the column changes with time.

A solution containing ten ions (K, Ti, Ca, Cr, Na, Zn, Al, Ni, Cu and Cd) at 170 ppb each was pressure injected at 5 p.s.i. s and run for 10 min at 15 kV with detection at 204 nm. Results are shown in Fig. 3. Several of the ions are not well separated, as expected from the migration time data in Table 1. The groups K/Ti, Zn/Na/Al and Cu/Cd are poorly resolved, but are easily detectable at 170 ppb.

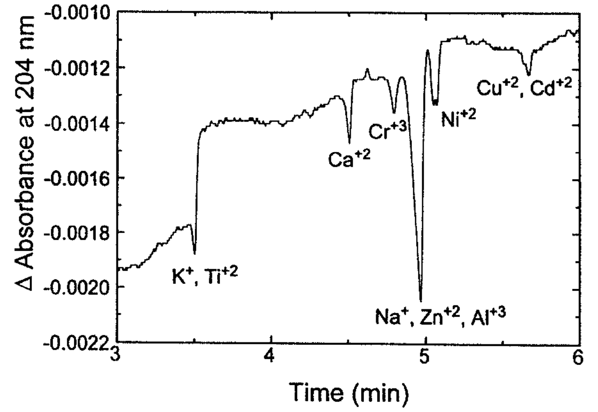

Fig. 3. Segment of electropherogram for a solution containing K, Ti, Ca, Cr Na, Zn, Ni, Al, Cu and Cd ions at 170 ppb. Electrolyte: 5.2 m*M* ephedrine, 4.7 m*M* HIBA, pH 2.8. Injection: 5 p.s.i. s. Run conditions: 15 kV, 10 min, 204 nm.

A new solution containing seven ions (Li, K, Ca, Cr, Zn, Al and Cu) at 200 ppb each was prepared and two runs were made for 10 min at 15 kV using two different methods of introducing the sample onto the column. Fig. 4 shows the result using a pressure injection of 5 p.s.i. s, and Fig. 5 shows the result using electro-injection at 10 kV for 10 s. The difference in peak sizes between the two runs reflects a difference in quantity of sample placed on the column by the two different injection methods. The full electropherogram from 0 to 6 min including the 3–6 min enlarged segment from Fig. 5 is shown in

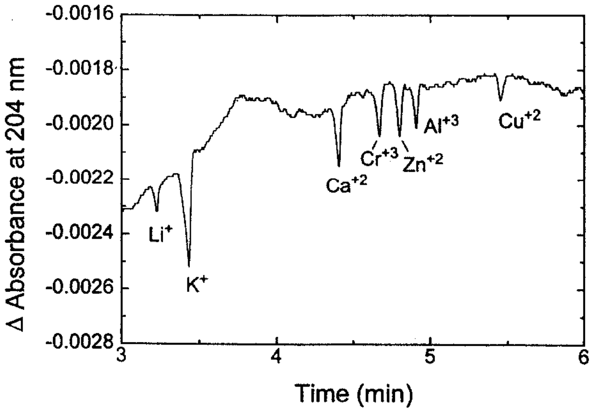

Fig. 4. Segment of electropherogram for a pressure-injected solution containing Li, K, Ca, Cr, Zn, Al and Cu ions at 200 ppb. Electrolyte: 5.2 m*M* ephedrine, 4.7 m*M* HIBA, pH 2.8. Injection: 5 p.s.i. s. Run conditions: 15 kV, 10 min, 204 nm.

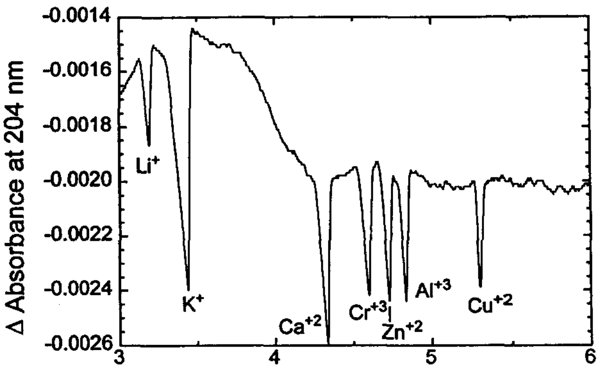

Fig. 5. Segment of electropherogram for an electro-injected solution containing Li, K, Ca, Cr, Zn, Al and Cu ions at 200 ppb. Electrolyte: 5.2 m*M* ephedrine, 4.7 m*M* HIBA, pH 2.8. Injection: 10 kV 10 s. Run conditions: 15 kV, 10 min, 204 nm.

Fig. 6 to indicate the relative magnitude of the acid peak to the peaks for ions of interest.

4. Discussion

4.1. Specificity and sensitivity

It can be seen from Figs. 4–6 that near the 200-ppb level, Al can be separated completely from Cu, Zn, Cr, Ca, K and Li ions, and that all these ions can be easily detected indirectly at 204 nm when using a background electrolyte containing 5.2 m*M* ephedrine and 4.7 m*M* HIBA at pH 2.8. However, migration times and mobilities listed in Table 1 indicate that Mg, Ni, Co, Mn and Na can be serious interferents that overlap the Al peak, especially if they are present in much greater concentration than the Al. All of the ions migrated past the detector window within 6 min at 15 kV and exhibited peaks that were nearly symmetrical. Data in Fig. 5 suggest that minimum detectable concentrations, based on peak height of twice the baseline noise, would lie near 50 ppb under the conditions used here.

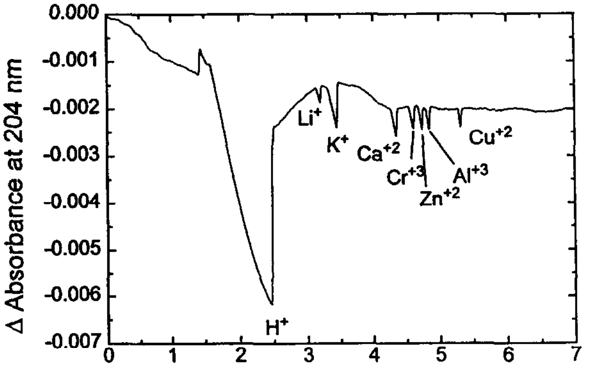

Fig. 6. Full electropherogram including Fig. 5 segment. Note the relative magnitude of the peak associated with high H^+ at low pH. (Same conditions as Fig. 5.)

From the spectrum of the background electrolyte (Fig. 1) it would be expected that sensitive detection could be achieved at any convenient wavelength in the range from 190 nm to about 206 nm. Weston *et al.* [7] have demonstrated that increasing the HIBA concentration increased the migration times of alkali, alkaline earth and transition metals. Therefore, some improvement in the separation of the Al from other specific ions might be achieved by adjustment of the HIBA concentration.

4.2. pH Dependence

The concentration of different Al species in solution depends critically on the solution pH. For example, consider the simple case of the aqueous solubility of $Al_2O_3 \cdot 3H_2O$ described by the following relationships [9]:

$$1/2\ Al_2O_3 \cdot 3H_2O(s) = Al^{3+} + 3OH^- \qquad \log K_a = -34 \tag{3}$$

$$1/2\ Al_2O_3 \cdot 3H_2O(s) + OH^- = Al(OH)_4{}^- \qquad \log K_b = -1 \tag{4}$$

$$H_2O = H^+ + OH^- \qquad \log K_w = -14 \tag{5}$$

By combining the expression for the water equilibrium constant, $[H^+] \times [OH^-] = 10^{-14}$, with each of the equilibrium constant expressions determined by Eqs. 3 and 4, expressions for the concentration of each soluble species as a function of pH are obtained. Fig. 7 was constructed to summarize the situation for this simple example that ignores complexities which result from the presence of additional ions. Diagrams of this

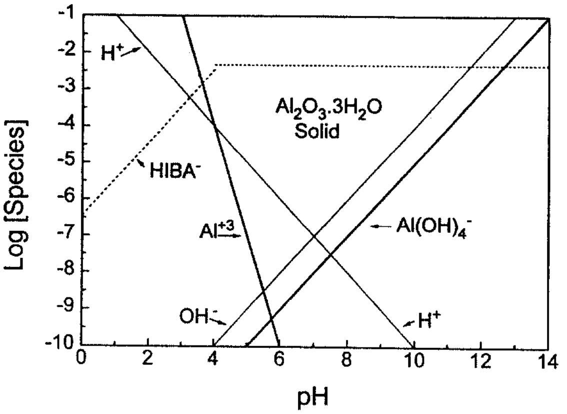

Fig. 7. Log (species concentration) *vs.* pH for an aqueous system containing $Al_2O_3 \cdot 3H_2O$ in equilibrium with Al^{3+}. At pH 5 a 2.7-ppb (10^{-7} *M*) Al^{3+} solution is saturated. Concentration of $HIBA^-$ in the $4.7 \cdot 10^{-3}$ *M* HIBA electrolyte is also shown ($HIBA = H^+ + HIBA^-$; p*K* = 3.717).

type are often used to describe the equilibria in natural water systems [1,9]. From Fig. 7 it is seen that minimum solubility of $Al_2O_3 \cdot 3H_2O$ occurs near pH 5.8. Therefore, to maintain Al^{3+} in solution and avoid losses of material during sample handling, even before sample injection, the pH must be maintained at a low value at all times. Fig. 7 also indicates that at pH 5 the concentration of Al^{3+} in equilibrium with $Al_2O_3 \cdot 3H_2O$ is near 10^{-7} *M* which is only 2.7 ppb. If the pH value approaches 5 or greater, excess Al^{3+} may precipitate and may also produce particles that can plug the capillary when the sample is injected into the column for analysis. Therefore, the background electrolytes were always adjusted to a pH less than 3, and during sample solution preparation the pH was never higher than 3.

To achieve reproducible analytical results, the pH of not only the run electrolyte, but also the analyte solution must be controlled. To investigate the effect of changing the pH of the analyte solution, a series of 5-ppm solutions of Al adjusted to different pH values were analyzed using the pH 2.8 run electrolyte containing 5.2 m*M* ephedrine and 4.7 m*M* HIBA. As a check against artifacts each solution was made independently rather than by adjusting the pH of aliquots of the same solution. Analytical conditions were as before: 5 p.s.i. s, 15 kV, 204 nm, 10 min. Results are shown in Fig. 8. There is not only a systematic variation of migration time with the pH change, but a significant change in peak shape. More acidic initial conditions result in longer migration times, larger prominent leading peaks, and an apparent shift in the fractional distribution of material from the low broad peak to the prominent leading peak. These effects are concentration dependent. The previously discussed 170- and 200-ppb solutions containing Al (pH≈2.8 when injected) have simple symmetrical peaks (Figs. 3–6), whereas peaks from the 25-fold more concentrated 5-ppm solutions are noticeably more complex. Working with electrolytes containing 6.5 m*M* HIBA in the pH range from 3.6 to 5, Weston *et al.* [7] also observed increased migration times with lower pH, and noted that reducing pH reduces the

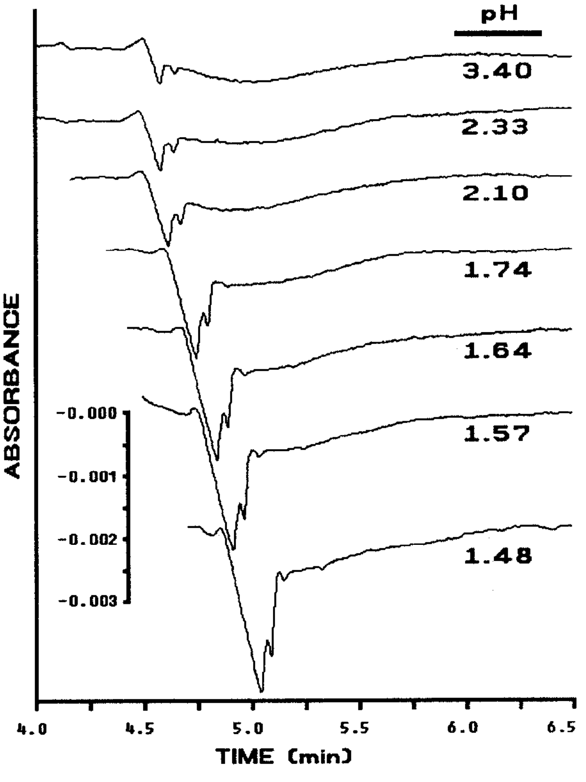

Fig. 8. Effect of pH of the analyte solution on migration time and peak shape of 5-ppm Al^{3+} solutions. Electrolyte: 5.2 m*M* ephedrine, 4.7 m*M* HIBA, pH 2.8. Injection: 5 p.s.i.s. Run conditions: 15 kV, 10 min, 204 nm.

effective concentration of the complex in the run electrolyte.

4.3. Extensions

This work describes an effective analytical protocol for the analysis of Al^{3+} ions in aqueous solutions by CZE. Future studies to select the best light-absorbing component for indirect or direct detection and to find the optimum combination of concentrations of the components of the electrolyte should result in improvement of the sensitivity and selectivity of this analytical technique.

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