Speciation of aluminum using capillary zone electrophoresis with indirect UV detection

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ABSTRACT

Capillary zone electrophoresis was evaluated as a method for the speciation of aluminum in aqueous solution using indirect UV absorption detection. Fluoro- and oxalatoaluminum complexes appeared as sharp and well-defined peaks within the electropherogram of aluminum standard solutions when the appropriate ligand was added. Siultaneous separation of these inorganic and organic aluminum species was achieved with good resolution in a single analytical run within 5 min. Indirect UV detection was achieved at 214 nm with a background electrolyte buffer containing 5 mM imidaxole. The average limit of detection was about 10 nM for uncomplexed and complexed aluminum species. Excellent agreement between experimental and theoretical species concentrations (via the thermodynamic speciation model SOILCHEM) was obtained for solutions with varying ligand/aluminum mol ratios and pH values.

INTRODUCTION

The role of aluminum (Al) as a toxic agent in neurodegenerative disorders such as Alzheimer's disease (AD) is a controversial but important topic. In recent years there is growing evidence which suggests that some chemical form(s) of Al could play an important role in governing the absorption and transport of Al in humans [1–5]. Unfortunately, very little is known about the concentrations and identities of Al species present in the environment and in biological systems. This problem is in large part due to the lack of analytical techniques capable of rapid and accurate determination of Al species present in various sample matrices.

Recently a controversial study which statistically links the ingestion of Al through drinking water and the increase frequency of AD has been reported [6]. Although in this study no evidence of direct relationship between Al and AD has been established, it points out the possibility that Al in drinking water may be present in some unique chemical form(s) that makes it more bioavailable than Al found in food stuffs or medications. In this regard it is interesting to note that among the potential inorganic ligands normally present in water, equilibrium calculations suggested that only the fluoride ion will significantly affect Al speciation [7]. The distribution of mononuclear fluoro-aluminum species, e.g., AlF^{2+}, AlF_2^+, AlF_3 and AlF_4^-, in water is of particular interest since some of these monomeric Al species have been postulated to facilitate the transport of hydrolyzable Al species through hydrophobic membranes and/or demonstrated to possess properties which alter biochemical pathways [8].

Early analytical methods employed for Al speciation mainly involved some type of indirect method [9]. The key step in all these methods was the use of cation-exchange columns to separate the labile monomeric (inorganic) from the non-labile monomeric (organic) fractions. Based on the concentration of the total monomeric Al,

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the labile monomeric concentration can then be calculated using thermodynamic-based speciation models. Recently direct determination of inorganic as well as organic Al species have been shown to be possible using ion chromatography (IC). For example, Bertsch and Anderson [10] have demonstrated that fluoro- oxalato-, and citratoaluminum complexes can be separated and quantitated by IC, and that excellent quantitative agreement between predicted species concentrations (calculated from thermodynamic speciation model) and those determined by IC were obtained under various experimental conditions. More recently, Jones [11] has applied an IC technique for the speciation of Al in reservoir and drinking water samples. In these studies major disadvantages of the use of IC methods for Al speciation included their inabilities to separate various inorganic or organic Al species with good efficiency and to resolve mixtures of inorganic and organic Al species into discrete chromatographic peaks under isocratic conditions.

In recent years capillary zone electrophoresis (CZE) has been shown to be a highly efficient technique for the separation of small inorganic as well as organic cations and anions. For example, simultaneous separation of cu. ten alkali, alkaline earth and lanthanide metal cations within a single run has been demonstrated using CZE [12]; also, the simultaneous analysis of weak organic acid anions (oxalate and citrate) and inorganic anions (chloride, sulfate, phosphate and carbonate) in diluted urine has been achieved within the same analytical run in a simple and rapid manner [13]. On the other hand, the use of CZE for the efficient separation of metal species with good resolution has also been shown to be feasible. For example, the determination of the oxyanionic species of arsenic, *i.e.,* arsenite and arsenate, in urine was demonstrated [13] and the separation of a mixture of metal complexes present in electroplating solutions, *i.e.,* $Fe(CN)_6^{4-}$, $Fe(CN)_6^{3-}$, $Cu(CN)_4^{3-}$ and $Zn(OH)_4^{2-}$ were also reported [14].

In this communication the separation performance of CZE for the speciation of **fluoro-** and oxalatoaluminum complexes present in aqueous solutions was evaluated. Detection was per-

formed using indirect absorption method with a background electrolyte containing imidazole as the UV absorber, which was first used by Beck and Engelhardt [15] for the rapid CZE determination inorganic cations and aliphatic **amines** with excellent detection limits and separation efficiencies. Furthermore, the stability of Al species during CZE separation was examined by comparing measured and predicted concentration values (via the thermodynamic speciation model: SOILCHEM) obtained for the various **fluoro-** and oxalatoaluminum complexes and the **uncomplexed** Al under various experimental conditions.

EXPERIMENTAL

Chemicals and materials

Aluminum nitrate, sodium fluoride, sodium oxalate and imidazole were analytical grade and purchased from Baker (Phillipsburg, NJ, USA) or Sigma (St. Louis, MO, USA). Doubly distilled deionized water obtained from a Barnstead Nanopure System (Dubuque, IA, USA) was used for the preparation of all buffer and sample solutions. Polyimide-coated fused-silica capillary with 75 μm I.D. and 360 μm O.D. was obtained from Polymicro Technologies (Phoenix, AZ, USA).

Buffer and sample solutions

All buffer solutions were prepared with a background electrolyte containing 5 **mM** of imidazole. Sample solutions were prepared by adding solution containing the ligand of interest to an appropriate volume of $Al(NO_3)_3$ standard solution in a **100-ml** polyethylene (PE) volumetric flask such that the final Al concentration was 0.25 **mM**. The **pH** of the buffer and sample solutions were adjusted with small amounts of 1 *M* sulfuric acid. Prior to use all solutions were filtered through a **0.45-μm** membrane filter and stored in the PE bottles to age for a minimum period of 24 h.

instrumentation

A home-built CZE instrument was used for all separations. An acrylic box with a safety-interlocked door designed to prevent operator con-

tact with high voltage contained the Speehnan voltage source (Model CZE 1000/30 PN, Plainview, NY, USA) and a Linear Instrument UV/Vis absorbance detector (Model 204, Reno, NV, USA) which has been modified for on-line detection at 214 nm. Contact with solution was made via platinum electrodes. All CZE separations were carried out using 350 V/cm field strength. Samples were injected hydrodynamically at the anode (positive polarity) by raising the sample solution reservoir to a height of 10 cm for 10 s. Data were recorded using a Chromjet integrator (Spectra Physics, San Jose, CA, USA).

Capillary preparation

Capillary length was 45 cm with a detection window made by burning off 2 mm of the polyimide coating at approximately 35 cm from the anode end of the capillary. Capillaries were washed with 1 M NaOH for 30 min, followed by rinsing with the buffer solution for 1 h and equilibration with the buffer solution overnight.

Speciation calculations

The theoretical species concentration of Al in the sample solutions was calculated by utilizing the SOILCHEM program: an improved version of the thermodynamic geochemical speciation model-GEOCHEM [16].

RESULTS AND DISCUSSION

Fig. la shows the CZE separation of a standard solution of $Al(NO_3)_3$ using a background electrolyte buffer containing 5 mM imidixole at pH 3.5, and two sharp and well-defined peaks can be observed in the electropherogram. The early peak which appears at a migration time of *ca.* 3.5 min can be assigned to the "free" Al ion (Al^{3+}) since this species predominates in aqueous solution at pH less than 5. This positively charged species would be expected to migrate faster toward the cathode (or detector) when compared to the predominant negatively charged solute present in the sample solution: NO;, which appears as an "inverted" peak at a migration time of *ca.* 6.5 min. Appropriate amount of nitric, hydrochloric and sulfuric acids were added to the buffer and sample solutions for pH adjust-
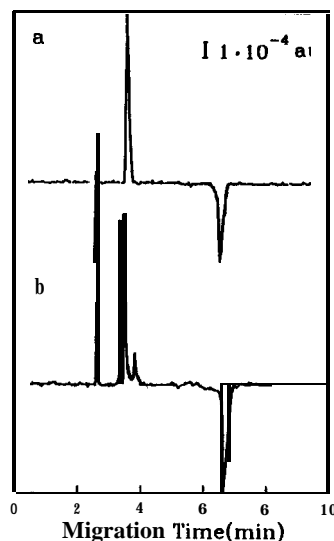

Fig. 1. CZE electropherogram of a 0.25 mM $Al(NO_3)_3$ solution with (a) no F and (b) the addition of 0.12 mM of NaF. The pH of buffer and sample solutions was matched at 3.5.

ments and it was found that separation performance was dependent on the type of acid used. Sulfuric acid was found to provide the best resolution for the CZE separation of various Al species, *vide infra,* but it is not clear at this time why this is the case. It should be noted that based on thermodynamic calculations, the SO_4^{2-} ion could interact with the Al ion to form sulfatoaluminum complexes in the sample solutions. However, by adding a relatively large amount of SO_4^{2-} to the Al standard solutions (mol ratio SO,: Al = 20:1), we found no significant difference in the retention time or peak area of the free Al ion peak as shown in Fig. la. This observation suggests that any sulfatoaluminum complexes formed in the sample solution are likely to exist as weak, electrostatic outer-sphere complexes and are apparently readily dissociated during CZE separation. Similar observations have also been reported for the separation of sulfatoaluminum complexes using IC [10].

Fig. lb shows that with the addition of F (as NaF) into the Al standard solutions, the peak area of the free Al ion peak was reduced significantly and additional peaks were produced in the CZE electropherogram. The major peak which appears at ca. 2.4 min can be assigned to

Na^+ [confirmed by the injection of **NaF** without the presence of $Al(NO_3)_3$]. On the other hand, assignments of the two smaller peaks which appear at cu. 3.2 and 3.8 min, respectively, are not as straightforward. Table I compares experimental data and theoretical values generated by the SOILCHEM program. At a F/Al ratio of 0.5:1 and sample and buffer **pH = 3.5** (experimental conditions of **Fig.** lb), the predicted concentration values of Al^{3+}, AlF^{2+} and AlF_2^+ are shown to be in good agreement with experimental measurements, which suggests the formation of AlF^{2+} and AlF_2^+ species upon the addition of **NaF** into the Al standard solution. Furthermore, it was found that the total integrated areas of the free Al ion as shown in Fig. la and the cluster of three adjacent peaks (between 3.0 and 4.0 min) as shown in Fig. lb are similar. These results, along with the fact that no interference peaks were obtained from the CZE separation of **NaF** alone, suggest that the two smaller peaks which center at cu. 3.2 and 3.8 min in Fig. lb are probably a result of a redistribution of Al between species of differing positive charges upon the addition of **NaF** and assignments of AlF^{2+} and AlF_2^+ could be made for these two additional peaks. In this case, it would be reasonable to assign AlF^{2+} as the early peak which appears at *ca.* 3.2 min since it possesses a higher positive charge and would be

expected to migrate faster toward the detector when compared to AlF_2^+, which was assigned as the latter peak appearing at cu. 3.8 min. However, using the same argument, it would be unreasonable to expect the Na^+ to migrate faster than AlF^{2+} as shown in Fig. lb and, similarly, the early appearance of AlF^{2+} when compared to the free 'Al ion (a positively triple charge species which appears at *ca.* 3.5 min) is not expected.

In an attempt to understand the migration order of the assigned peaks as shown in Fig. lb, it is important to note that the mobility (or migration time) of a particular ion in CZE separation is dependent not only on the charge but also the size of the ion [17]. For electrolytes dissolved in aqueous solutions the number of water molecules associated with the ions during their movement through the solution is likely to influence their overall size and mobility. Many experiments have been devised in attempts to determine the hydration of ions in aqueous solutions [18,19]. Unfortunately, the solvation (or hydration) number of many ions is not known with certainty. However, it is interesting to note that under the influence of intense electrical forces, the hydration numbers of **NaCl** and $AlCl_3$ have been reported to be 7 and 31, respectively [18]. It is possible that under the experimental conditions described in Fig. lb, the mobilities of

TABLE I

MEASURED (CZE) AND PREDICTED (SOILCHEM) CONCENTRATIONS OF UNCOMPLEXED Al AND COMPLEX FLUOROALUMINUM SPECIES IN SOLUTIONS OF VARYING F:Al MOL RATIOS AND pH VALUES

F/Al"	pH	Al^{3+} (mM)			AlF^{2+} (mM)			AlF_2^+ (mM)		
		CZE[b]	CZE[c]	SOILCHEM	CZE[b]	CZE[c]	SOILCHEM	CZE[b]	CZE[c]	SOILCHEM
0.5:1	3.2	0.143	0.141	0.127	0.104	0.101	0.115	0.003	0.008	0.005
	3.5	0.129	0.134	0.132	0.115	0.111	0.113	0.006	0.005	0.005
	4.0	0.135	0.130	0.122	0.112	0.114	0.124	0.003	0.006	0.004
	4.2	0.132	0.137	0.130	0.113	0.108	0.114	0.005	0.005	0.006
1:1	3.2	0.075	0.073	0.058	0.138	0.148	0.144	0.037	0.029	0.048
	3.5	0.068	0.069	0.059	0.150	0.149	0.145	0.031	0.032	0.046
	4.0	0.066	0.069	0.060	0.155	0.156	0.148	0.029	0.025	0.042
	4.2	0.075	0.069	0.059	0.139	0.139	0.147	0.036	0.041	0.044

[a] Total Al concentration = 0.25 mM.
[b] pH of the sample and buffer solutions was matched in the range of 3.2 to 4.2.
[c] pH of the buffer was fixed at 3.5 and pH of the sample solutions was adjusted from 3.2 to 4.2.

the various ions are influenced by the degrees of hydration. The hydration number of the un-complexed Al ions may be higher than that of Na^+ and AlF^{2+}, thus leading to an increase in the size and/or decrease of the charge of the free Al ion and resulting in a longer migration time.

Table I and Fig. 2 show that good agreement between measured and predicted values extend over a range of pH and mole ratio values when the pH of the sample and background electrolyte buffer were matched. On the other hand, when the pH of the sample and buffer was unmatched, the experimental values of uncomplexed Al and complexed fluoroaluminum species showed minimal changes and also exhibit good agreement with thermodynamic calculated values over a pH range of about one order of magnitude. These results suggest that these fluoroaluminum species exhibited good kinetic stability and maintained their integrity, *i.e.,* minimal redistribution during CZE separation, under the present experimental conditions. The average limits of detection (LOD) for uncomplexed Al and complexed fluoroaluminum species was found to be about 10 nM (SIN = 3) and the average relative standard deviations (n = 8) for mobilities and peak areas were <0.5% and 4%, respectively. The

relationship between peak area and concentration was linear in the range 10 to 500 nM with the regression equation: A (peak area) = 1.04 · $10^4 C$ (concentration) + 1.09 · 10^3. The correlation coefficient (r) was 0.998 (n = 8).

Fig. 3 shows the CZE separation of a sample solution containing mixed F and oxalate ligands (mole ratio Al:F:oxalate = 1:0.5:1). When compared to Fig. 1b, an additional peak which appears at cu. 4.0 min was produced with the presence of oxalate anion. Similarly, a peak which appears at cu. 4.0 min was also produced upon the addition of oxalate into Al standard solutions without the present of F. Fig. 4 compares the experimental and thermodynamic calculated values of uncomplexed Al and mono-oxalatoaluminum species at varying mole ratio of oxalate and Al (in the absence of F) and indicates good agreement for these particular species, suggesting that that additional peak at cu. 4.0 min probably arose from the formation of monooxalatoaluminum species. The relatively long migration time obtained for this organic Al species is reasonable since it would have the smallest charge density due to its single positive charge and relatively large size (neglecting hydrating number), thus leading to lower mobility relative to the -other positively charged species
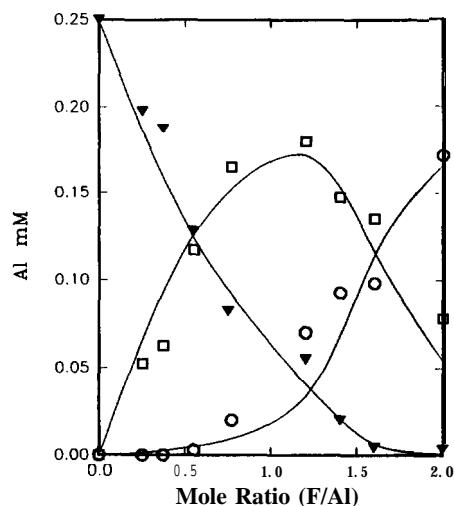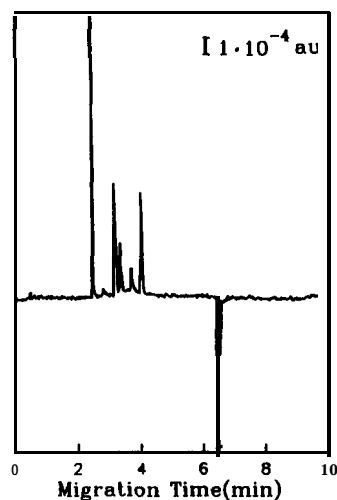

Fig. 2. Measured (symbols) and predicted (solid lines) concentrations of the uncomplexed and fluoro-complexed Al species at varying F:Al mol ratios with total Al concentration equal to *0.25* mM and pH of buffer and sample solutions equal to 3.5. ▼ = Al^{3+}, □ = AlF^{2+} and 0 = AlF_2^+.

Fig. 3. CZE electropherogram of a 0.25 mM $Al(NO_3)_3$ solution with the presence of 0.13 mM F and 0.25 mM oxalate. The pH of buffer and sample solutions was matched at 3.5.
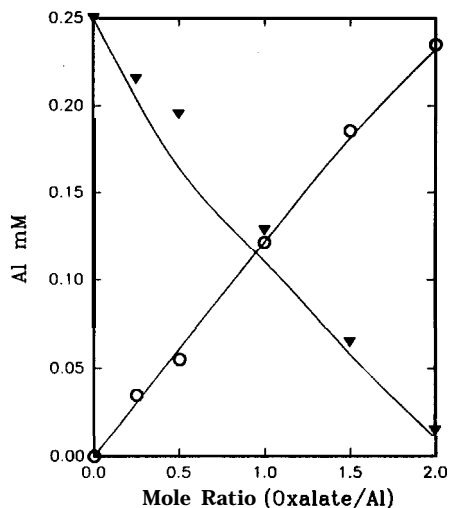
Fig. 4. Measured (symbols) and predicted (solid lines) concentrations of the **uncomplexed** and monooxalato-complexed Al species at varying oxalate: Al ratios with total Al concentration equal to 0.25 **mM** and **pH** of **buffer** and sample solutions equal to 3.5. ▼ = Al^{3+}, 0 = Al-oxalate'.

present in the sample solution. Fig. 3 shows that good resolution can be obtained for the simultaneous separation of inorganic and organic Al complexes within a single CZE run during a period of less than 5 min. This capability demonstrates that CZE possesses a distinct advantage over IC for the separation of sample solutions containing these particular mixed inorganic and organic ligands. Using IC only the **uncomplexed** Al and AlF^{2+} produced discrete **chromatographic** peaks while AlF_2^+ and **monooxalatoaluminum** species were observed to co-elute as a single peak under isocratic conditions [10].

In summary, this work demonstrates that CZE can be used as a powerful technique for the rapid and efficient separation of fluoro- and **oxalato**-aluminum species present in aqueous solutions. More research is needed to examine the feasibility of using CZE for the separation of other small inorganic and organic Al complexes, as well as protein-bound and macromolecular Al species. In particular, experimental factors which affect the stability or integrity of the Al species during

CZE separation and the effects of potential interference ions present in real samples which might form complexes with aluminum should be investigated in detail by comparing experimental and theoretical values.

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