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Capillary zone electrophoretic and micellar electrokinetic capillary chromatographic separations of polyaminopolycarboxylic acids as their copper complexes

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Abstract

Capillary zone electrophoretic (CZE) and micellar electrokinetic capillary chromatographic (MECC) separations with UV absorbance detection at 290 nm were developed for separation and detection of the negatively-charged copper complexes of the polyaminopolycarboxylic acids. A standard mixture that contained ethylenediaminetetraacetic (EDTA), nitrilotriacetic (NTA), ethylenediaminetriacetic (ED3A), diethylenetriaminepentaacetic (DTPA) and hydroxyethylenediaminetriacetic (HEDTA) acids was utilized to demonstrate these separation methods. MECC techniques were performed with either cationic (cetyltrimethylammonium bromide, CTAB) or anionic (sodium dodecyl sulfate, SDS) surfactants. All three separation techniques achieved separation of the standard components with baseline resolution. A fast separation (7.5 min) was obtained with CTAB surfactant that provided a detection limit of 38 pg for HEDTA. The cationic micellar strategy was also extended to separations of mixed-metal EDTA complexes.

Keywords: Environmental analysis; Complexation; Polyaminopolycarboxylic acids; Carboxylic acids; Copper complexes; Detergents; Metal complexes

1. Introduction

Trace analytical method development is required for environmental studies of polyaminopolycarboxylic acids. Commercial detergent use has resulted in ubiquitous release of polyaminopolycarboxylic acids (principally EDTA and NTA) to the environment [1,2]. Environmental persistence and fate questions have been raised because of the ability of these compounds to mobilize toxic metals. Trace analytical methods are also needed to monitor leakage of defense waste from underground storage tanks. This waste contains large quantities of polyaminopolycarboxylic acids that were used for decontaminating plutonium processing facilities. The presence of polyaminopolycarboxylic acids in this waste increases the probability of subsurface aquifer contamination, since complexed radionuclides migrate through the soil at enhanced rates compared to their noncomplexed counterparts [3]. Chelation may also enhance the propensity for plant uptake and bioaccumulation of radionuclides and/or toxic metals [4–6]. High-resolution separation techniques that operate on small sample volumes are also needed to delineate the fate of polyaminopolycarboxylic acids in defense waste where high radiation fluxes lead to unusual reactions.

Polyaminopolycarboxylic acids are often analyzed by derivatization followed by capillary gas chromatography (GC) or by paired-ion high-performance

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liquid chromatography (HPLC). For GC analysis, methyl esters are formed by reaction with boron trifluoride–methanol prior to analysis by high-resolution capillary GC [7]. The derivatization approach has several problems including erratic methylation of some chelator fragments (most notably the ethylenediaminediacetic acid isomers) and possible formation of artifacts and by-products [8]. Alternatively, polyaminopolycarboxylic acids may be separated as their negatively-charged copper complexes (pH 5.5) on a reversed-phase HPLC column with a mobile phase containing copper(II) and a quaternary amine paired-ion reagent [9–11]. Although this method is robust, the technique suffers several constraints including limited resolution, relatively large sample volume requirements, the necessity for lengthy mobile phase equilibration and the production of large quantities of waste associated with the separation.

A variety of studies have examined the electroseparation of intact polyaminopolycarboxylic acid–metal complexes. These studies typically separate metal ions as the metal chelates in the presence of excess ligand. Experimental approaches range from paper electrophoresis [12] to capillary electroseparation techniques including isotachophoresis [13–15] and CZE [16,17]. One strategy is to form metal chelates with EDTA or one of its analogues (i.e., 1,2-cyclohexanediamine-N,N,N′,N′-tetraacetic acid). The UV-absorbing properties of these ligands allow low wavelength detection of the complexed metals.

The goal of this study was to develop microcolumn zone electrophoretic and micellar separation techniques for the analysis of polyaminopolycarboxylic acids. Our approach was based on the formation of copper(II) complexes followed by capillary electroseparation of the negatively-charged complexes. Capillary electroseparation of the copper-complexed polyaminopolycarboxylic acids has not been extensively investigated. Similar methods were explored for extending these studies to the MECC separation of mixed-metal EDTA complexes.

2. Experimental

The electroseparation system used for the present study was assembled in-house as previously described [18]. Fused-silica capillaries (0.8 m×50 μm I.D.×360 μm O.D.), obtained from Polymicro Technologies (Phoenix, AZ, USA), were preconditioned by treatment with 0.1 *M* NaOH followed by a water rinse before equilibration with separation buffer. Detection windows were placed approximately 20 cm from the ground reservoir on these columns. Buffer reservoirs were contained within a Lexan box fitted with a safety interlock system to protect the operator from high-voltage contact. High-voltage and ground connections were provided through platinum electrodes immersed in the buffer reservoirs. Voltage was delivered to the capillary injection reservoir by a Spellman CZE 1000R power supply (Plainview, NY, USA). A Keithly 175A autoranging multimeter (Cleveland, OH, USA) was placed in series between the detector reservoir and ground to measure separation current. Introduction of samples to the separation capillary was accomplished hydrodynamically. An Isco-CV[4] capillary electrophoresis absorbance detector (Lincoln, NE, USA) was utilized for analyte detection. The detector was operated at 290 nm for copper-complexed polyaminopolycarboxylic acids or at 220 nm for separations of mixed-metal EDTA complexes. Chromatographic traces were recorded on a Hewlett-Packard Model 3393A integrator (Avondale, PA, USA).

The polyaminopolycarboxylic acid sodium salts, with the exception of DTPA and ED3A, were obtained from Aldrich (Milwaukee, WI, USA). DTPA was obtained from the same manufacturer as the free acid. ED3A was synthesized by reaction of ethylenediamine-N,N′-diacetic acid and chloroacetic acid (both from Aldrich) according to the procedure of Genik-Sas-Berezowsky and Spinner [19]. After precipitation of EDTA from the acidified reaction mixture, ED3A lactam was purified from other reaction by-products by differential solubility in dimethylformamide. Product identity and purity of ED3A lactam were verified by combined capillary gas chromatography–mass spectrometry of the methyl ester.

Cobalt(II) EDTA was prepared according to the procedure of Weakliem and Hoard [20]. Cobalt(III) EDTA was prepared from the Co(II) EDTA product by oxidation with hydrogen peroxide. EDTA complexes of Fe(III) and Cr(III) were synthesized as described by Lind et al. [21] and Hamm [22], respectively.

High-purity copper(II) acetate monohydrate (99.99+%) was purchased from Aldrich. Cetyltrimethylammonium bromide (CTAB) and dodecyltrimethylammonium bromide (DTAB) surfactants were provided by Sigma (St. Louis, MO, USA). Electrophoresis-grade sodium dodecyl sulfate (SDS) was obtained from Bio-Rad (Richmond, CA, USA). Glacial acetic acid was sequencing grade (aldehyde free) supplied from FisherBiotech (Fair Lawn, NJ, USA). Electroseparation buffers were prepared from HPLC-grade water obtained from J.T. Baker (Phillipsburg, NJ, USA).

Electroseparation buffers were prepared from a stock solution that contained 0.10 *M* $Cu(OAc)_2$ and 0.175 *M* acetic acid. CZE buffer consisted of 10.0 ml concentrated stock solution, 100 μl glacial acetic acid and approximately 90 ml water. The solution was adjusted to a pH of 5.5 with sodium hydroxide before dilution to 100.0 ml with water. Preparation of micellar phases was similar except 300 μl acetic acid was incorporated in these buffers and 5.0 ml rather than 10.0 ml of stock solution was utilized for the anionic surfactant buffer. CTAB (3.64 g/100 ml) and SDS (2.16 g/100 ml) surfactants were dissolved before pH adjustment. The buffer used for separation of mixed-metal EDTA complexes was prepared by adding 200 μl acetic acid and 3.08 g DTAB to 90 ml of water, adjusting the pH to 5.5 and bringing the final volume to 100.0 ml. All buffers were filtered through 0.45-μm filters (Millex–LCR, Millipore, Bedford, MA, USA) prior to use. Polyaminopolycarboxylic acid standards (approximately 1 mg/ml) were prepared either individually or in various combinations in ten-fold diluted copper acetate stock solution. Mixed-metal EDTA samples were prepared in DTAB separation buffer at mg/ml concentrations.

3. Results and discussion

3.1. CZE separations

CZE separations used columns that were first preconditioned with 0.1 *M* NaOH for 1 h, rinsed with water and then equilibrated overnight with separation buffer. The CZE buffer consisted of 10 m*M* $Cu(OAc)_2$ and 35 m*M* HOAc at pH of 5.5. An electropherogram of five standard copper chelates is given in Fig. 1. For this separation, a −30 kV voltage

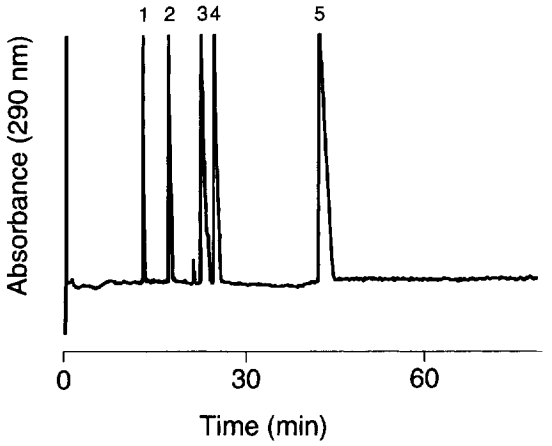

Fig. 1. Separation of copper complexes of polyaminopolycarboxylic acids by CZE. Peak identities are: (1) $[Cu(II)EDTA]^{-2}$, (2) $[Cu(II)NTA]^{-1}$, (3) $[Cu(II)HEDTA]^{-1}$, (4) $[Cu(II)_2DTPA]^{-1}$ and (5) $[Cu(II)ED3A]^{-1}$.

applied to the injection reservoir resulted in a separation current of −45.83 μA. The electropherogram (Fig. 1) illustrates baseline resolution of the model components within 45 min. In order of increasing migration times the $[Cu(II)EDTA]^{-2}$ complex eluted first, followed by $[Cu(II)NTA]^{-1}$, $[Cu(II)HEDTA]^{-1}$, $[Cu(II)_2DTPA]^{-1}$ and finally $[Cu(II)ED3A]^{-1}$. Although migration times were somewhat variable, analyte identity was readily established by co-injection experiments.

CZE migration times are a composite of electrophoretic migration and electroosmotic flow. With conventional CZE buffers, mobile cations contained in the Stern-layer are repelled by the positive injection electrode resulting in an electroosmotic flow toward the detector. It is known that divalent copper can be incorporated, either ionically or perhaps even covalently, into the fused-silica surface although the exact nature of this association remains poorly understood [23]. The presence of divalent cations and/or cationic surfactants alters the Stern-layer structure which may result in a diminished magnitude (or even reversal) of electroosmotic flow. To obtain the CZE separation shown in Fig. 1, it was necessary to impose a negative voltage on the injection reservoir; this resulted in an electroosmotic flow toward the negative electrode. Negatively-charged polyaminopolycarboxylic acid–copper complexes were repelled from the negative injection

electrode and therefore displayed an electrophoretic migration toward the detector. The polyaminopolycarboxylic acid–copper complexes migrated faster than, and in an opposite direction to, the electroosmotic flow. EDTA, being the most highly charged of the complexes, exhibited the highest electrophoretic mobility and was the first complex to elute from the column. Counter-current migration of the copper complexes accounted for the relatively long electrophoretic migration times.

Although this separation was reproduced several times with different fused-silica capillaries, several column pretreatments and/or buffer equilibrations were often required before acceptable separations were obtained. Lack of reproducibility was attributed to variability in electroosmotic flow. Subtle differences in fused-silica stock, column sample history, column pretreatment and buffer equilibration may result in slight Stern-layer alterations and hence different electroosmotic flow characteristics. Previous investigators have encountered similar difficulties when using copper-containing electroseparation buffers [23,24]. These studies point to meticulous capillary column pretreatment [24] and capillary copper loading regimes [23] as key steps in obtaining reproducible separations. Lengthy analysis times along with limited understanding of capillary preparation techniques required for producing highly reproducible separations, preclude routine implementation of the polyaminopolycarboxylic acid CZE method at this time.

Further work, focused on establishing reproducible separation conditions for this relatively simple buffer system, may prove important based on interface compatibility considerations with certain selective detectors. For example, studies performed by Deacon and co-workers [23] have demonstrated highly selective and sensitive detection by oxidative carbon-fiber amperometric detection in the presence of copper(II)-containing buffers. Other intriguing possibilities exist such as on-column formation of copper complexes in capillaries that have been previously saturated with Cu(II). Conceivably, polyaminopolycarboxylic acids could be introduced, derivatized in situ, separated as negatively-charged copper complexes and detected by electrospray ionization or fast atom bombardment mass spectrometry. Ideally, this approach would minimize mass spectrometer fouling since copper-containing buffers may no longer be necessary, as the capillary surface will liberate only the precisely required quantity of copper necessary to complex the analytes. Due to the expected low copper background, this approach would also be ideal for inductively coupled plasma mass spectrometric detection [25].

3.2. MECC separations

3.2.1. Cationic surfactant micelles for polyaminopolycarboxylic acid separations

Micellar separations were investigated with the objective of obtaining reproducible separations within short time periods. Cationic micellar systems were initially studied due to the expected electrostatic interaction between the positively-charged micelles and the negatively-charged analyte copper complexes. These separations used a buffer consisting of 100 m*M* CTAB, 10 m*M* $Cu(OAc)_2$ and 70 m*M* HOAc at a pH of 5.5. Due to electroosmotic flow reversal caused by cationic surfactant inclusion, negative voltage was applied to the injection reservoir to maintain buffer flow toward the detector. Fig. 2 illustrates a representative separation of the model polyaminopolycarboxylic acid–copper complexes. This separation was obtained with an applied voltage of −20 kV which resulted in a separation current of −37.17 μA. In this separation, the $[Cu(II)ED3A]^{-1}$

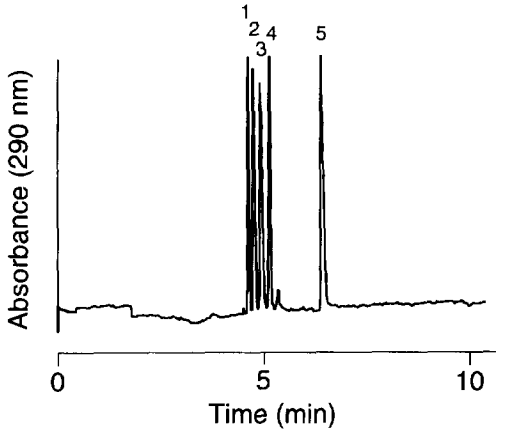

Fig. 2. Separation of copper complexes of polyaminopolycarboxylic acids by MECC using CTAB as surfactant. Peak identities are: (1) $[Cu(II)ED3A]^{-1}$, (2) $[Cu(II)_2DTPA]^{-1}$, (3) $[Cu(II)HEDTA]^{-1}$, (4) $[Cu(II)NTA]^{-1}$ and (5) $[Cu(II)EDTA]^{-2}$.

chelate eluted first, followed by $[Cu(II)_2DTPA]^{-1}$, $[Cu(II)HEDTA]^{-1}$, $[Cu(II)NTA]^{-1}$ and finally $[Cu(II)EDTA]^{-2}$. This elution order was exactly opposite to that previously observed during CZE separation. The electrostatic interaction between the micelles and the copper chelates proved to be a highly selective separation mechanism as indicated by the baseline resolution of the polyaminopolycarboxylic acids. In this system, micelles slowly migrate toward the injection reservoir countercurrent to a strong electroosmotic flow. Retention is determined by analyte affinity for the micelle balanced by the opposing migration of free metal complex in solution toward the detector. Since the micelle aggregation number of CTAB is relatively large (n_{aggr}=61) [26], charge alteration associated with analyte interaction is likely to have a negligible effect on micelle mobility. As the most highly charged species, $[Cu(II)EDTA]^{-2}$, is retained the longest, it appears that on balance, micelle affinity is the dominating effect rather than the higher electrophoretic mobility of this metal chelate in solution.

The cationic surfactant separation is potentially very useful due to the high resolution and the short analysis times. Complete separation of the model components was achieved within 7.5 min. The resulting electrophoretic zones were extremely sharp which, consequently, allowed for exceptional analyte detection sensitivity. Injections of dilute HEDTA solutions allowed determination of the analytical detection limit. For this study, 3.49 nl injections of 33.8 ppm Na_3HEDTA (118 pg) were introduced to the column. Calculations based on the detector response indicated that 38 pg Na_3HEDTA (110 femtomole) would give a signal of three times noise. Although detection limits were not determined for each individual polyaminopolycarboxylic acid, it can be assumed that the other chelators would exhibit roughly similar sensitivities. Further improvements in detection limits may be possible by substituting alternative detection modes such as amperometric [27,28] or radioisotopic [29,30] detection.

In efforts to further improve the resolution of the above separation, we attempted to perform MECC in a buffer prepared with ^2H_2O. Enhanced electrophoretic resolution in ^2H_2O-based buffers over their aqueous counterparts has been attributed to a lower electroosmotic flow [31,32]. The lower electroosmotic flow is a consequence of the lower ionization constant and higher viscosity of ^2H_2O. For the cationic micellar system, separation in a corresponding ^2H_2O-based buffer did not prove feasible due to the inevitable precipitation of CTAB surfactant. Precipitation of buffer components was observed only rarely in the H_2O-based system.

3.2.2. Anionic surfactant micelles for polyaminopolycarboxylic acid separations

A micellar system that incorporated an anionic surfactant was also investigated for separation of the polyaminopolycarboxylic acids. This micellar electroseparation buffer contained 75 m*M* SDS, 5 m*M* $Cu(OAc)_2$ and 35 m*M* HOAc at a pH of 5.5. MECC was effected by imposing a +30 kV voltage to the injection reservoir, which resulted in a separation current of +72.62 μA. A representative separation of the model chelates with the SDS micellar system is presented in Fig. 3. This separation provided baseline resolution of the polyaminopolycarboxylic acids within a 25-min time frame. The elution of analytes in increasing order of migration time was $[Cu(II)ED3A]^{-1}$, $[Cu(II)_2DTPA]^{-1}$, $[Cu(II)HEDTA]^{-1}$, $[Cu(II)NTA]^{-1}$ and finally $[Cu(II)EDTA]^{-2}$. This elution order was identical to that observed for cationic MECC separations and exactly opposite of the CZE elution order.

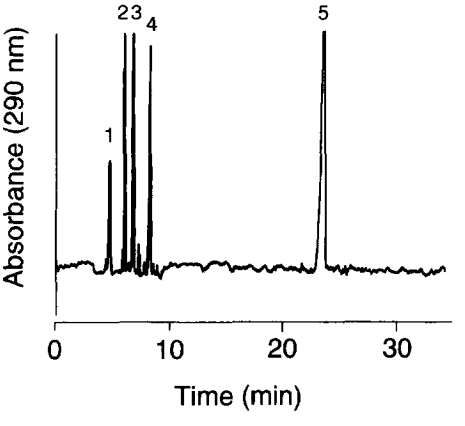

Fig. 3. Separation of copper complexes of polyaminopolycarboxylic acids by micellar electrokinetic capillary chromatography using SDS as surfactant. Peak identities are: (1) $[Cu(II)ED3A]^{-1}$, (2) $[Cu(II)_2DTPA]^{-1}$, (3) $[Cu(II)HEDTA]^{-1}$, (4) $[Cu(II)NTA]^{-1}$ and (5) $[Cu(II)EDTA]^{-2}$.

One might expect that the anionic micellar buffer would be a poor medium for analyte discrimination based on nonspecific charge repulsion between the negatively-charged copper-complexed analyte and the anionic surfactant micelles. However, the separation (Fig. 3) clearly involves a highly selective mechanism as evidenced by baseline resolution of the model constituents. The probable separation mechanism involves interaction of the micelle and the copper-complexed polyaminopolycarboxylic acids through a copper(II) bridge. It has been hypothesized that similar interactions are responsible for unique specificities observed between SDS micelles and oligonucleotides in the presence of various divalent cations [33].

3.2.3. Cationic surfactant micelles for separation of mixed-metal EDTA complexes

We pursued development of separations for mixed-metal EDTA complexes as an extension of the above separation principles. A desirable separation would retain the high resolution and rapid analysis characteristics of the cationic micellar separation. However, unlike the cationic micellar separation for polyaminopolycarboxylic acids, the separation buffer for mixed-metal EDTA complexes should be free of divalent cations to avoid perturbing metal chelate equilibria. Initial experiments focused on separating a mixture of Fe(III), Co(III), Cr(III) complexes of EDTA. The electrophoretic buffer investigated was a 70 m*M* acetate buffer at pH of 5.5 that contained 100 m*M* DTAB. A voltage of −20 kV imposed on the injection reservoir resulted in a separation current of −42.02 μA. A representative separation that illustrates baseline resolution of the model components within 6 min is presented in Fig. 4. This system allowed rapid and efficient separation of simple solutions of mixed-metal EDTA complexes. However, as metal EDTA sample complexity increased, multiple unknown peaks began to appear in the electropherograms due to complex and competing equilibria. Other limitations were also apparent with this electroseparation system. Analysis of solutions containing Co(II) EDTA did not result in an identifiable peak. It appears that electroseparation conditions promote decomposition of this metal chelate, perhaps by promoting oxidation of the metal center. Therefore, oxidation–reduction reactions may be a further complicating factor that must be considered.

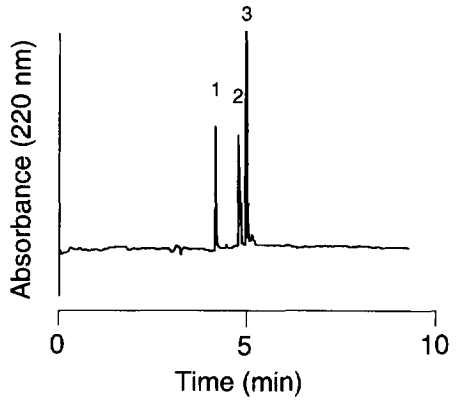

Fig. 4. Separation of mixed-metal EDTA complexes of (1) $[Fe(III)EDTA]^{-1}$, (2) $[Cr(III)EDTA]^{-1}$ and (3) $[Co(III)EDTA]^{-1}$ by micellar electrokinetic capillary chromatography in a buffer containing DTAB as surfactant.

4. Conclusion

Capillary electroseparation techniques were developed to allow determination of polyaminopolycarboxylic acids as their negatively-charged copper complexes. Of the CZE and MECC techniques investigated, the micellar approaches appeared more generally suitable for analysis of polyaminopolycarboxylic acids. MECC separation based on CTAB surfactant provided extremely high separation efficiencies, low detection limits (38 pg for HEDTA) and rapid analysis times (within 7.5 min). The SDS micellar system also provided separations within a reasonable time frame (within 25 min). Sequential application of the anionic and cationic surfactant separations provides a powerful tool for identification of polyaminopolycarboxylic acids. Tentative identification of copper chelates can first be assigned by separation on one system and then verified by retention characteristics on the second system. Due to the unique selectivities of the individual electroseparation systems, co-elution of an unknown with an analytical standard on both systems would constitute good evidence of compound identity. Switching between the two micellar systems is a simple operation that involves capillary column pretreatment with NaOH followed by column

equilibration with the alternate buffer. Additional experiments were conducted that demonstrated an electroseparation of mixed-metal EDTA chelates and highlighted several limitations associated with this analysis.

Capillary separation techniques have several potentially useful properties for the analysis of defense waste. These separations allow reduced radiation exposure and minimal production of separation-related radioactive waste due to the small injection volume requirements and low flow-rates. Additionally, the characteristic high separation efficiencies associated with microcolumn separation techniques allow complete resolution of individual components in complex samples. However, injection of samples with higher conductivity than the electroseparation buffer is normally not feasible due to solute band defocusing which occurs upon application of separation voltage [34]. Since waste samples have extremely high ionic strength, it is likely that sample pretreatment will be required to reduce both ionic strength and radionuclide content before capillary electroseparation techniques can be successfully applied.

Capillary techniques are also ideally suited for trace analytical environmental studies due to the enhanced mass detectability observed with concentration-dependent detectors [28] and compatibility with unique miniaturized detection devices [26,27,29,35] as well as certain mass spectrometric interfaces [36,37]. Although impressive detection limits were obtained by UV absorbance of the copper complexes (38 pg), substitution of more sensitive detectors may be possible. For example, oxidative amperometric detection for selective detection of polyaminopolycarboxylic acids in the presence of buffer constituents is a possibility. Preliminary studies (data not shown) were performed that demonstrated the feasibility of separating polyaminopolycarboxylic acids as their negatively-charged Co(II), rather than Cu(II), complexes under both CZE and micellar conditions. Sensitive detection of the polyaminopolycarboxylic acids could be accomplished by formation and separation of the ^{56}Co(II) complexes. Electrolyte background signal would be extremely low since only nonradioactive isotopes of cobalt would be used to prepare the separation buffer. Provided polyaminopolycarboxylic acid complexes were formed with high-specific-activity ^{56}Co, radioisotopic detection could be expected to be quite sensitive as well as highly selective. Polyaminopolycarboxylic acid analysis based on capillary electroseparation of the ^{59}Fe(III) complexes followed by radioisotopic detection also is a possibility that warrants investigation.

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