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Separation of uranium(VI) and transition metal ions with 4-(2-thiazolylazo)resorcinol by capillary electrophoresis

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Abstract

A capillary electrophoresis method utilizing 4-(2-thiazolylazo)resorcinol (TAR) was developed to separate uranium, cobalt, cadmium, nickel, titanium and copper metal ions. TAR was chosen as the visible absorbing chelating ligand because of its ability to form stable complexes with a wide variety of metals. Several parameters that included pH, electrophoretic run buffer concentration, buffer type and the influence of chelating ligand in the electrophoretic run buffer were examined to determine the best separating conditions. Optimum separation of the six metal chelates was achieved in a 15 mM $\text{Na}_2\text{B}_4\text{O}_7\text{-NaH}_2\text{PO}_4$, pH 8.3 buffer containing 0.1 mM TAR. Method validation included injection and method precision studies as well as detection limit and linear dynamic range determination. High-ppb to low-ppm (w/w ratio) detection limits were achieved with linear dynamic ranges between 0.1 and 75 ppm. © 2001 Published by Elsevier Science B.V.

Keywords: Metal chelates; Buffer composition; Uranium; Transition metal; Thiazolylazoresorcinol

1. Introduction

Capillary electrophoresis (CE) continues to play an important role in the analytical development of new techniques for the determination of metal ions in solution [1]. Interest stems from the high separation efficiency, low absolute detection limits and differential selectivity achievable by CE when compared to other analytical approaches. The US Navy has a number of important metal monitoring needs, including bilge water analysis for preventing environmental

contamination of waterways at various ports of call, and monitoring hull scrubbing operations for the protection of estuaries from high levels of copper emanating from paints. We are also interested in the detection of radionuclides in surface and groundwater, in particular, uranium, in order to assist the US Government's efforts to monitor remediation efforts at contaminated Superfund waste sites. Because of their instrumental similarities, CE is an excellent proving ground for developing analytical methodologies amenable to CE glass microchips, a rapidly evolving field which lends itself well to the type of portable field sensing requirements mandated by these monitoring programs [2,3]. In this paper, we will focus on the CE characterization of 4-(2-thiazolylazo)resorcinol (TAR), a strong metal complexing colorimetric agent, for the CE detection of uranium and various transition metal cations.

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Metal ion separation and quantitation by CE have been achieved using both (1) weak metal complexation agents, wherein a substantial part of the metal exists as an uncomplexed cation and separations are attained due to a secondary complexation effect superimposed upon the cation migration, e.g., 2-hydroxyisobutyric acid (HIBA) [4] and crown ethers [5] in the indirect absorption detection of alkali and alkaline earth metal ions, and (2) strong metal complexation agents, wherein nearly all of the metal is complexed and electrophoretic separation of the complexed metal ion must be achieved, e.g., 4-(2-pyridylazo)resorcinol (PAR) [6] and 2-(5-bromo-2-pyridylazo)-5-(diethylamino)phenol (PAD-AP) [7] in the direct absorption detection of transition metal ions. Although strong metallochromic chelators offer the potential for significantly improved sensitivity due to their high molar absorptivities and large colorimetric shifts, separations can be more difficult due to the similarity in size and charge of the various metal complexes.

While CE has been used extensively in the separation of various transition and alkali/alkaline earth metal ions, the separation and detection of uranium by CE has only received limited attention [1]. Of the few CE examples that do exist, most have relied on the colorimetric metal complexation dye, arsenazo III, which has proven to be very sensitive to uranium and the lanthanide metal ions [8–10]. Arsenazo III, however, has only limited colorimetric complexation of transition metal ions, a property which limits its applicability in some applications. Similar comments can be made with regard to the ligand, 2-[(2-arsenophenyl)-azo]-1,8-dihydroxy-7-[(2,4,6-tribromophenyl)azo]naphthalene-3,6-disulfonic acid which was utilized by Liu et al. for the CE separation of thorium, uranium and rare earth elements [11]. Other available CE methods for the detection of uranium have been based upon ligands such as cyclohexane-1,2-diaminetetraacetic acid (CDTA) [12] or 2,6-diacetylpyridine bis(*N*-methylene-pyridiniohydrazone) [13], both of which depend upon UV detection at either 214 or 254 nm, respectively. We are specifically seeking ligands, though, which absorb at more red-shifted wavelengths of the visible light spectrum, in order to take advantage of compact, inexpensive light sources such as diode lasers or light emitting diodes (LEDs). Timerbaev et

al. briefly mentioned the use of PAR in an attempt to detect uranium, but found that the peak shape was broad, asymmetric and the intensity too weak to include in their electropherograms [9].

TAR is a direct analogue of PAR, with the difference being the replacement of the pyridylazo moiety with a thiazolylazo moiety. Separations of strongly complexed PAR metal chelates are achievable due to the differential influence complexed metal ions have upon the pK_a of the *p*-phenolic group of this ligand [1]. TAR, due to its structural similarity, will likely give similar separation capabilities. Various successful extraction and spectrophotometric applications based upon uranium(VI) complexation by TAR encouraged us that TAR might prove to be a useful ligand for enabling the simultaneous separation of both uranium and transition metal ions by CE with detection capabilities in the visible (~530 nm) [14,15]. To our knowledge, there have been no published reports of the application of TAR as a strong metal complexation ligand in the separation of metal ions via CE. In this paper, we describe the effect of pH, buffer type and electrophoretic run buffer concentration on the separation of TAR metal chelates. In addition, we report linearity and precision data used to validate the separation of uranium and several transition metals.

2. Experimental

2.1. Reagents

TAR was purchased from Aldrich (Milwaukee, WI, USA). Atomic absorption metal reference standards from either Sigma (St. Louis, MO, USA) or Aldrich were used to make the metal stock solutions. All buffers were prepared from sodium tetraborate (borax, $\text{Na}_2\text{B}_4\text{O}_7$), sodium phosphate monobasic (NaH_2PO_4) or sodium phosphate dibasic (Na_2HPO_4). Deionized water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Sample solution preparation

A $5 \cdot 10^{-3}$ M stock solution of TAR was prepared in 15 mM NaH_2PO_4 – $\text{Na}_2\text{B}_4\text{O}_7$ buffer, pH 8.3. Stock solutions of cobalt, copper, cadmium, nickel,

titanium and uranium were prepared by dilution of the metal standards in water. Pre-capillary ligand–metal complexation was achieved by reacting the appropriate amount of metal ion with 1 mM TAR prepared from the stock solution. NaOH or HCl was used to adjust the pH of the samples to 8.3, after which the solutions were filtered through a 2- μm membrane filter (ChromTech, Apple Valley, MN, USA) and allowed to react for 5 min prior to injection.

2.3. Instrumentation

All experiments were performed on a BioFocus 3000 CE system (Bio-Rad, Hercules, CA, USA) equipped with a 72 cm effective length \times 50 μm I.D. fused-silica capillary (Alltech, Deerfield, IL, USA). Samples were hydrostatically injected into the capillary for 2 s, after which the separation was performed in a normal polarity mode at +25 kV. Detection was achieved at the cathodic end with a photo-diode array detector functioning in either the single wavelength (530 nm) or scanning mode (370–600 nm). Instrument control and data processing were accomplished using BioFocus and Spectra software, versions 3.00 and Integration software, version 3.01.

2.4. Separating procedure

New capillaries were initially conditioned with 15 mM $\text{Na}_2\text{B}_4\text{O}_7$ (pH 12) for 30 min, followed by a 30-min rinse with deionized water. To ensure reproducibility, all experiments were performed at 20°C and were run in triplicate. Before each separation, the capillary was rinsed for 1 min with 15 mM $\text{Na}_2\text{B}_4\text{O}_7$ (pH 12) buffer, followed by a 2-min rinse with deionized water and finally equilibrated with the run buffer for 2 min. Rhodamine B (Lambda Physik, Bedford, MA, USA) was used as the neutral marker to measure the electroosmotic flow (EOF).

2.5. Method validation

Linearity and precision studies were done to validate the capillary zone electrophoretic (CZE) separation method. Method linearity was evaluated for each of the TAR–metal complexes injected

separately. Metal concentrations ranged from 0.1 ppm to 75 ppm (w/w ratio) in a 15 mM NaH_2PO_4 – $\text{Na}_2\text{B}_4\text{O}_7$ buffer, pH 8.3. Injection precision was evaluated by three successive injections of a solution of the cobalt complex (5.0 ppm). Method precision was evaluated by preparing three individual solutions of the nickel complex (2.5 ppm) and of the cadmium complex (5.0 ppm) and injecting each of the six samples once.

3. Results and discussion

3.1. TAR background and spectra

Shown in Fig. 1 are the spectra for TAR and its metallochromic complexes formed with cadmium(II), copper(II) and uranium(VI). The remaining transition metals studied here were omitted for clarity and due to similarity in spectral shape. From an optical detection standpoint, TAR benefits from the capability for colorimetrically chelating a number of different transition and heavy metal cations, in

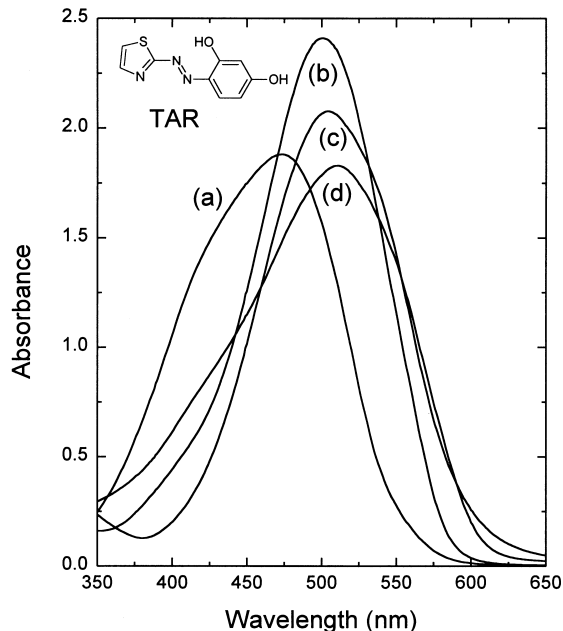


Fig. 1. Absorbance versus wavelength spectra for (a) 0.068 mM TAR and 0.049 mM (b) cadmium (II)–TAR, (c) copper (II)–TAR and (d) uranium (VI)–TAR complexes, measured in a 15 mM NaH_2PO_4 – $\text{Na}_2\text{B}_4\text{O}_7$ buffer adjusted to pH 8.5.

addition to uranium(VI), with high molar absorptivities [e.g., U(VI)–TAR, $\epsilon=2.1 \cdot 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ at 530 nm] and large bathochromic shifts [e.g., U(VI)–TAR, $\Delta=37 \text{ nm}$].

For comparison purposes, PAR deprotonates from pH 6–11 to a univalent anion (HL^-) which, in general, complexes divalent metal cations in a terdentate fashion with a 2:1 ligand to metal ratio, leaving the metal complexes uncharged and seemingly inseparable by CE [16]. Iki et al., however, demonstrated the separation of four transition metal ions using PAR, and postulated that the $\text{p}K_a$ of the *p*-phenolic group of PAR is influenced by the central metal ion, thereby enabling their separation by CE [17]. We can further postulate that the metal ion complexes successfully separated in this application likely have some equilibrium of positively charged 1:1 ligand to metal ion complexes. TAR is well studied in terms of its reactivity with uranium and various transition metal ions, and has similar complexation properties to PAR [18]. The slight differences in their reactivity toward metal ions are largely due to their structural disparities [19]. TAR also behaves as a terdentate ligand, but binds uranium and most transition metals in predominantly a 1:1 stoichiometric ratio [19]. An exception is made when complexing with Cu^{2+} where a 2:1 ratio persists. This raises the possibility that TAR may be much better suited for separating metal ions than PAR (in the absence of a surfactant). Micellar electrokinetic chromatography (MEKC) has in fact been successfully applied to PAR CE separations in order to increase the number of metal cations simultaneously determined from four to nine [9]. MEKC enables the separation of both neutral and charged species via the addition of surfactants to the background electrolyte (BGE), resulting in the formation of charged micelles that can be separated by the applied field. The addition of surfactant was not pursued as a viable option here due to the discovery that the uranium peak height decreased with increasing concentration of sodium dodecyl sulfate (SDS).

3.2. Nature of electrophoretic run buffer

As a suitable starting point for optimizing the CE separation of TAR and its metal chelates, the phos-

phate–borate buffer successfully utilized by Iki et al. in the non-micellar CE separation of four PAR metal chelates was investigated [17]. Initial electropherograms obtained in the separation of copper, nickel and uranium [using a 10 mM NaH_2PO_4 – $\text{Na}_2\text{B}_4\text{O}_7$ buffer (pH 8.3) containing 0.1 mM TAR in the run buffer solution and 1 mM TAR in the sample solution] gave peak to peak resolutions which were greater than 3.0, and efficiencies exceeding 120 000 for most of the metal complexes. Because of this success, a phosphate–borate buffer system was pursued further for optimization appropriate to TAR and its metal chelation properties.

3.3. Effect of electrophoretic run buffer concentration

Higher concentrations of electrophoretic run buffer solution have been shown to reduce the zeta potential, electrical double layer, and EOF (*u*) [20]. In addition, the adsorption of electrolytes onto the capillary walls can be effectively prevented, thus increasing the separation efficiency and resolution [21]. We found that resolution increased as a function of the electrophoretic run buffer concentration for each of the metal complexes. However, a steady-state was reached at 15 mM for several of the complexes, whereas the resolution continued to increase for the others (Fig. 2). When the electrophoretic run buffer concentration was 5 mM, the resolution between the TAR complexes of copper/cadmium and cadmium/nickel were zero, indicative of co-elution. Increasing the electrophoretic run buffer concentration improved the separation of the metal chelates, but at the expense of longer run times. For example, the uranium(VI) complex eluted several minutes earlier when separation was conducted in a 15 mM rather than a 20 mM electrophoretic run buffer concentration. The resolution for the early eluting peaks, e.g., cobalt/free TAR, and late eluting TAR complexes of nickel/titanium and titanium/uranium continued to increase beyond 15 mM. However, the resolution for free TAR/copper, copper/cadmium, and cadmium/nickel reached a steady state, implying that the resolutions for the previously mentioned early and late eluting complexes are more sensitive to the concentration or ionic strength of the electrophoretic run buffer. Although

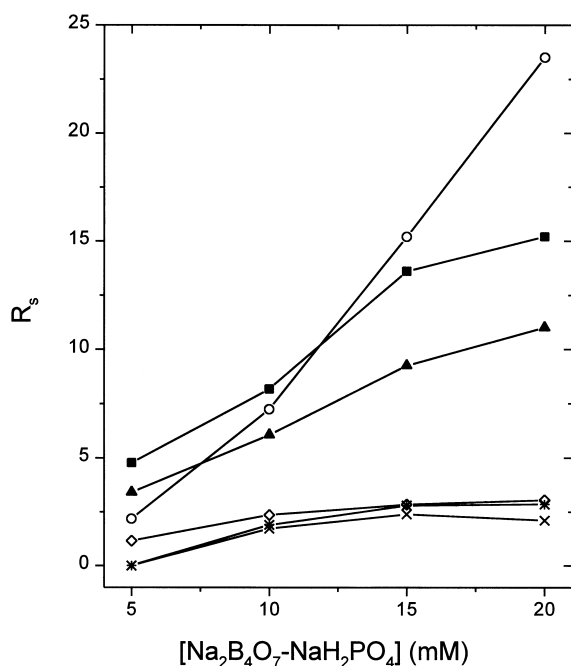


Fig. 2. Effect of electrophoretic run buffer concentration on resolution between two adjacent peaks. Electrophoretic run buffer: NaH₂PO₄-Na₂B₄O₇, pH 8.3, 1·10⁻⁴ M TAR. Titanium/uranium (○); cobalt/TAR (■); nickel/titanium (▲); TAR/copper (◇); cadmium/nickel (*); copper/cadmium (×).

resolution was enhanced at 20 mM, the optimal electrophoretic run buffer concentration chosen was 15 mM, due to the shorter migration times.

3.4. Effect of electrophoretic run buffer pH

The pH of the electrophoretic run buffer must be carefully controlled, as it not only influences the EOF, but also the acid dissociation equilibria of the TAR metal complexes, ultimately affecting sensitivity and resolution. The effect of pH on the mobility of several metal complexes is illustrated in Fig. 3. With the exception of uranium(VI), the electrophoretic mobilities (EOMs) were found to decrease as the pH of the electrophoretic run buffer increased. The titanium complex was not detectable when the electrophoretic solution was buffered at either pH 6.5 or 7.5. Nickless et al. discovered that TAR forms weaker complexes under acidic to slightly alkaline conditions, which explains the absence of a titanium

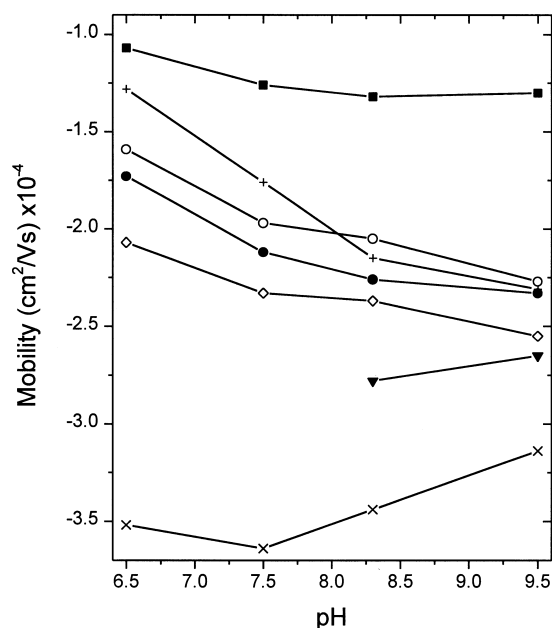


Fig. 3. Influence of pH on the electrophoretic mobility of the metal-TAR complexes. Electrophoretic run buffer: 15 mM NaH₂PO₄-Na₂B₄O₇, 1·10⁻⁴ M TAR. Cobalt (■); copper (+); TAR (○); cadmium (●); nickel (◇); titanium (▼); uranium (×).

TAR complex in the electropherogram under these conditions [18]. Decreases in EOMs were most significant between pH 6.5 and 8.3, especially for the copper-TAR complex. This pH region overlaps the pK_a of the *para*-hydroxyl group in the copper-TAR complex [17]. As the pH is decreased below the pK_a for a particular metal complex, we would expect an increase in the equilibrated concentration of doubly charged cations (assuming a 1:1 ligand to metal ratio), i.e., [M-TAR]²⁺, which will elute faster under the positive potential applied than the singly charged complexes predominating at pH values above the pK_a, i.e., [M-TAR]⁺. A similar phenomenon was reported earlier by Iki et al. for the separation of various metal-PAR complexes [17]. TAR complexes will tend to have slightly lower acid dissociation constants when compared to PAR, due to the acidic nature induced by the thiazole sulfur atom [19]. For those complexes which did not report significant decreases in EOMs with increasing pH, e.g., UO₂²⁺ and Co²⁺, the acid dissociation constants likely do not overlap this pH region.

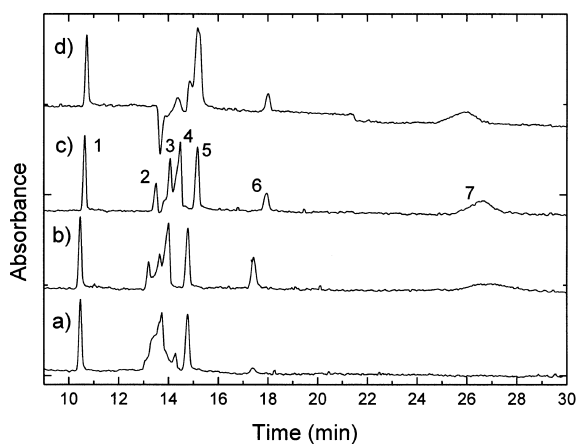


Fig. 4. Electropherograms of metal-TAR complexes at various concentrations of TAR in 15 mM NaH_2PO_4 - $\text{Na}_2\text{B}_4\text{O}_7$, pH 8.3, electrophoretic run buffer: (a) 0.0 mM, (b) 0.05 mM and (c) 0.10 mM, (d) 0.5 mM. 1=Cobalt (5 ppm), 2=free TAR, 3=copper (5 ppm), 4=cadmium (5 ppm), 5=nickel (2.5 ppm), 6=titanium (15 ppm) and 7=uranium (30 ppm).

3.5. Effect of adding chelating ligand to the electrophoretic run buffer

Shown in Fig. 4 is the effect of adding TAR to the electrophoretic run buffer with respect to the sensitivity and resolution of six metal chelates, Co^{2+} , Cu^{2+} , Cd^{2+} , Ni^{2+} , Ti^{4+} , and UO_2^{2+} . A lack of TAR in the electrophoretic run buffer resulted in a dramatic reduction of the uranium and titanium peaks, along with significantly impaired resolution of the copper and cadmium complexes. The cobalt and nickel TAR complexes, on the other hand, remained relatively unaffected. Because the thermodynamic stability constants for each of these metal complexes are comparable, we can conclude that it is the kinetic lability or inertness of these TAR complexes which dictates whether the TAR metal complex can survive the column separation length without dissociating and becoming undetectable. Uranium, of course, has the longest transit time, and, therefore, has a significantly increased probability for dissociating prior to reaching the detector.

The kinetic lability of metal complexes formed with strong metal complexation agents is a common problem encountered in the CE analysis of metals, and is typically dealt with via the addition of the chelating ligand to the background electrolyte of the

buffer as well as the sample pre-complexing solution [1]. Increasing the TAR concentration in the buffer adjusts the equilibrium in favor of the metal complex, and ensures that if the metal ligand complex should dissociate and become spatially separated, additional TAR ligands within the buffer will maintain the metal's detectability. As the concentration of TAR in the buffer becomes too high, however, two detrimental effects occurred: (1) the signal-to-noise ratio diminished drastically making the detection of several metal complexes impossible (especially at 1 mM or higher levels of TAR, data which is not shown in Fig. 4), and (2) the introduction of a large, negative TAR peak which severely impacts the resolution of several of the metal ions. The optimal concentration of chelating ligand in the buffer necessary to attain the highest level of resolution and sensitivity was concluded to be 0.1 mM.

3.6. Method validation

Separation of the metal complexes was optimal in a 15 mM phosphate-borax buffer (pH 8.3) containing 0.1 mM TAR. Fig. 5 shows an electropherogram of the cobalt, copper, cadmium, nickel, titanium and uranium complexes, all of which were well separated.

Injection precision was evaluated for the cobalt complex (5.0 ppm) using the optimized conditions.

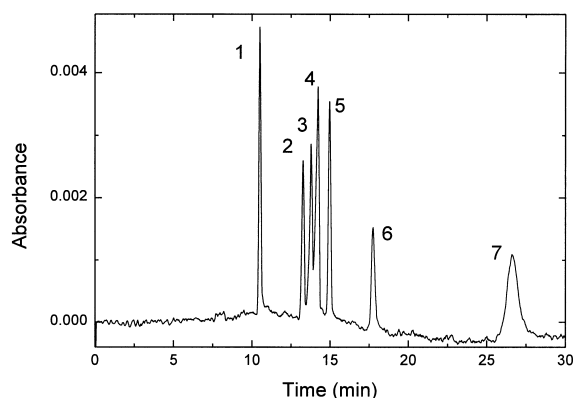


Fig. 5. Separation of TAR complexes in 15 mM NaH_2PO_4 - $\text{Na}_2\text{B}_4\text{O}_7$, pH 8.3, $1 \cdot 10^{-4}$ M TAR (optimum conditions). 1=Cobalt (5 ppm), 2=free TAR, 3=copper (5 ppm), 4=cadmium (5 ppm), 5=nickel (2.5 ppm), 6=titanium (15 ppm) and 7=uranium (30 ppm).

Table 1
Detection limits and range of linearity

Metal	Detection limit	Range of linearity (ppm)
Cobalt	88.0 ppb	0.2–35.0
Cadmium	114 ppb	0.3–50.0
Nickel	59.0 ppb	0.1–43.0
Copper	144 ppb	0.3–25.0
Titanium	733 ppb	2.0–50.0
Uranium	1.70 ppm	3.0–75.0

The relative standard deviations (RSDs) of four successive injections of a 5.0 ppm solution of the cobalt complex were 0.71 and 0.84%, for peak area and peak height, respectively. Method precision was evaluated for individual solutions of the nickel (2.5 ppm) and cadmium (5.0 ppm) complexes. Method precision for the nickel complex was 1.9% RSD using peak area and 2.9% RSD using peak height analysis. Method precision for the cadmium complex was 5.2% RSD using peak area and 5.4% RSD using peak height analysis.

At a 3:1 signal-to-noise ratio, the cobalt complex exhibited a detection limit of 88 ppb. The detection limits of the other metal complexes varied up to 1.7 ppm for uranium. The linear dynamic range of each metal complex was determined from the analysis of several standards of varying concentrations. Table 1 lists the detection limits and linear dynamic range of each metal complex.

4. Conclusions

We have demonstrated the first application of TAR to CE in the successful separation of six metal ions. TAR is unique with respect to PAR in its ability to detect uranium(VI) simultaneous with other transition metal ions. Efforts are currently underway to transfer this method to a microchip device for improved portability and response time. Improvements in response time by as much as 50 times are feasible when converting to a microchip platform, and by utilizing the steady light output of an LED, we predict an improvement in sensitivity by at least three times. Because of the large difference in retention times obtained for uranium and the preceding metal ion, titanium, larger sample plugs may be

loaded onto the microchannel in order to further enhance the sensitivity to uranium(VI).

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References

- [1] M. Macka, P.R. Haddad, *Electrophoresis* 18 (1997) 2482.
- [2] Q. Lu, J.H. Callahan, G.E. Collins, *Chem. Commun.* 19 (2000) 1913.
- [3] G.E. Collins, Q. Lu, *Sensors Actuators B* (2001) in press.
- [4] A. Weston, P.R. Brown, P. Jandik, W.R. Jones, A.L. Heckinberg, *J. Chromatogr.* 593 (1992) 289.
- [5] Y. Shi, J.S. Fritz, *J. Chromatogr. A* 671 (1994) 429.
- [6] T. Saitoh, H. Hoshino, T. Yotsuyanagi, *J. Chromatogr.* 469 (1989) 175.
- [7] D.A. Oxspring, R.J. Maxwell, W.F. Smyth, *Anal. Proc.* 32 (1995) 489.
- [8] B.A. Colburn, M.J. Sepaniak, E.R. Hinton, *J. Liq. Chromatogr.* 18 (1995) 3699.
- [9] A.R. Timerbaev, O.P. Semenova, P. Jandik, G.K. Bonn, *J. Chromatogr. A* 671 (1994) 419.
- [10] M. Macka, P. Nesterenko, P. Andersson, P.R. Haddad, *J. Chromatogr. A* 803 (1998) 279.
- [11] B. Liu, L. Liu, J. Cheng, *Anal. Chim. Acta* 358 (1998) 157.
- [12] A.R. Timerbaev, O.P. Semenova, J.S. Fritz, *J. Chromatogr. A* 756 (1996) 300.
- [13] A.R. Timerbaev, O.P. Semenova, G.K. Bonn, J.S. Fritz, *Anal. Chim. Acta* 296 (1994) 119.
- [14] R. Saraswati, T.H. Rao, *J. Liq. Chromatogr.* 16 (1993) 1601.
- [15] C.H. Lee, M.Y. Suh, J.S. Kim, D.Y. Kim, W.H. Kim, T.Y. Eom, *Anal. Chim. Acta* 382 (1999) 199.
- [16] K.L. Cheng, K. Ueno, T. Imamura (Eds.), *CRC Handbook of Organic Analytical Reagents*, CRC Press, Boca Raton, FL, 1992, p. 195.
- [17] N. Iki, H. Hoshino, T. Yotsuyanagi, *Chem. Lett.* 4 (1993) 701.
- [18] G. Nickless, F.H. Pollard, T.J. Samuelson, *Anal. Chim. Acta* 39 (1967) 37.
- [19] R.W. Stanley, G.E. Cheney, *Talanta* 13 (1966) 1619.
- [20] J. Knox, *J. Chromatogr. A* 680 (1994) 3.
- [21] D.S. Burgi, R. Chien, *Anal. Chem.* 63 (1991) 2042.