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Metal speciation by means of microbore columns with direct-injection nebulization by inductively coupled plasma atomic emission spectroscopy

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ABSTRACT

Ion chromatography can be used to perform speciation of several elements. This paper examines the use of ion chromatography with inductively coupled plasma atomic emission spectroscopy detection. A new type of nebulizer, the direct-injection nebulizer, was used to introduce the sample into the plasma. Columns were developed to interface with the direct-injection nebulizer at a flow-rate of 80–100 μl/min. The speciation of arsenic, selenium and chromium is discussed.

INTRODUCTION

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) is often used to determine the concentrations of elements in a sample. However, the total elemental concentration is not a complete description because an element is often present in a variety of chemical forms or species. Different species of an element may possess quite different biological, medicinal or toxicological properties. For example, Cr(VI) is extremely toxic, but a trace amount of Cr(III) is essential for human health. Other elements exhibit similar characteristics. Measuring the concentration of the different chemical forms of a particular element can be important to determine the hazards of a particular sample.

The work presented here describes general methods for elemental speciation using microbore columns. Ion chromatography has been successful in separating the various species of particular elements. Because sample matrices are often complex and the elements present in low concentrations, selective detection may be needed to achieve the desired sensitivity.

ICP-AES has been interfaced with liquid chromatography for elemental detection [1]. A nebulizer introduces the column effluent into the ICP. Conventional pneumatic nebulizers operate at about 1 ml/min sample flow. This is compatible with conventional ion chromatography columns. However, this type of nebulizer may introduce as little as 1% of the sample into the plasma.

This work utilizes the direct-injection nebulizer (DIN) developed by workers at Iowa State Univer-

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sity [2–8]. This nebulizer operates at much lower sample flow-rates (5–10% of that normally used for conventional pneumatic nebulizers). By design, 100% of the sample is introduced into the ICP. This results in significant advantages including superior precision, elimination of memory effects, higher sample throughput, improved detection limits and elimination of speciation effects. Another advantage of the DIN interface with chromatographic columns is that a large range of separation conditions can be used without any need to adjust the nebulizing conditions. Anion-exchange, cation-exchange, reversed-phase, size-exclusion columns, etc. are easily switched without changing nebulizer interface parameters. For conventional nebulizers, each eluent may require a modification of the sample introduction conditions to maintain a stable plasma. Chromatographic buffer gradients are difficult to perform with conventional nebulizers. Moreover, special conditions must be used to introduce organic solvents with conventional nebulizers, otherwise the plasma could extinguish [9]. The DIN will tolerate eluent gradients up to 100% organic solvents with no change in operating conditions. This is due to the low flow-rates used and the fineness of the DIN aerosol.

Where possible, the separation conditions were based on those developed for standard bore columns (4.0–4.6 mm I.D.) [10]. However, the DIN was designed to accommodate a column effluent input rate of 10–100 μl/min. The microbore columns developed for this work require eluent flow-rates of about 100 μl/min. Eluent flow-rates of *ca.* 100 μl/min rather than 10 μl/min were used in order to introduce the maximum amount of sample to the plasma and gain the greatest sensitivity. Typical column diameters ranged from 1.6 to 2.0 mm I.D. and column lengths ranged from 2 to 15 cm.

EXPERIMENTAL

Ultrex-II-grade nitric acid was from J. T. Baker. Trace metal-grade ammonium hydroxide was from Fisher, and the organoarsenic species were from Aldrich. All other chemicals used were reagent grade or better.

All columns (Sarasep) were manufactured using polyether ether ketone (PEEK) or polyethylene frits, tubes and endfittings. All columns were packed with macroporous, strong-base anion exchangers with an exchange capacity of 0.05 mequiv./g. Column lengths were chosen based on the efficiency needed to separate a particular mixture. If only two species were to be separated, then the shortest possible column was selected to give a 1–2-min separation. If more species were to be separated or the sample matrix contained a high concentration of ions, then a longer column was used. The column hardware was connected directly to the inlet of the DIN–ICP-AES via a short length of 0.3 mm I.D. PEEK tubing. The columns used are summarized in Table I.

TABLE I

SUMMARY OF MICROCOLUMNS USED FOR SPECIATION

Mercury, lead, tin and some arsenic speciation work performed by Shum and co-workers [11,12] on Sarasep-supplied columns.

Species	Column
As(III), As(V)	ANX1705
As(III), DMA, MMA, As(V)	ANX1710, RPX1710
Hg^{2+}	RPX1705
Hg^{2+}, methyl-Hg^{+}, ethyl-Hg^{+}	RPX1715
Pb^{2+}, trimethyl-Pb^{+}, triethyl-Pb^{+}	RPX1705, C181705
Alkyl tin species	RPX1710
Se(IV), Se(VI)	ANX1705
Cr(III), Cr(VI)	ANX1710

In cases where few components in a mixture were to be separated and high sensitivity was desired, large injections of 5–20 μl were used. In cases where the sample contained a high ionic matrix or several species to be separated, smaller injection volumes of 0.2–0.5 μl can be used to gain the greatest column resolution. A Rheodyne Model 9010 injector was used in all work.

The DIN used was a System 3 from Cetac Technologies, and the HPLC pump used was a Model 222D from Scientific Systems. Sample traveled through a fused-silica capillary of 50 μm I.D. (Fig. 1). A concentric tube was used to introduce argon gas which nebulized the sample into the plasma. The ICP spectrometer used was a Thermo Jarrell Ash ICAP 61. Operating conditions for the DIN and the ICP spectrometer are given in Table II.

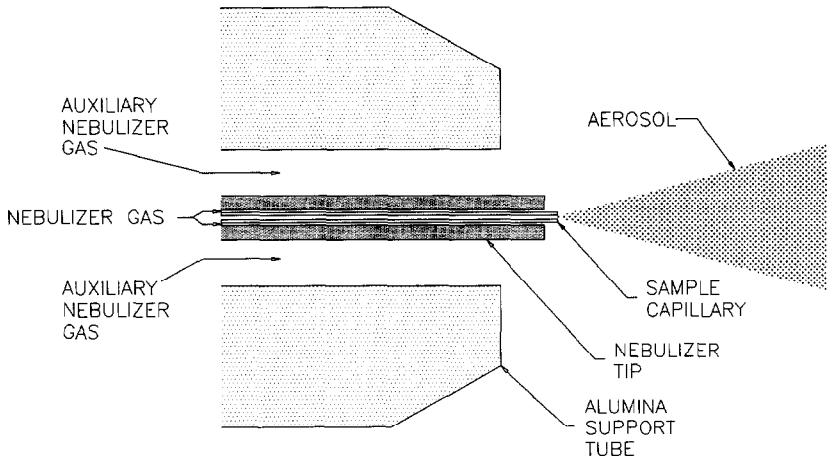

Fig. 1. Schematic of direct-injection nebulizer (DIN). The complete aerosol is directed into the ICP.

RESULTS AND DISCUSSION

Arsenic

Arsenic is very common and is found in soil, preservatives, coal, etc. As(III) and As(V) are particularly toxic. Organoarsenic species appear to be less toxic. Previous DIN interface work performed by Shum and co-workers [11,12] demonstrated that reversed-phase microbore columns and eluents containing ion-pairing reagents could be coupled with mass spectrometric (MS) detection for arsenic speciation.

Much of the previous speciation work with standard columns has been with anion-exchange type columns [13–17]. In this work, a microbore column (10 cm × 1.7 mm I.D.) packed with low-capacity anion-exchange material (0.05 mequiv./g capacity) was used for the speciation of As(III), monomethylarsonate and As(V) (Fig. 2) The eluent contained 5 m*M* ammonium carbonate and 5 m*M* ammonium bicarbonate at pH 8.6. The ammonium salts were used to prevent salt formation and plugging of the DIN tip. A relatively large injection volume (10 μl) was used to obtain higher sensitivity. Dimethylarsinate (DMA), if present, would elute directly after As(III) and not be completely resolved. A smaller injection volume (0.5 μl) or a longer column would be needed to resolve DMA and As(III). If the pK_a values of the species to be separated differ, adjusting the eluent buffer pH can improve the resolution of

TABLE II

DIN AND ICP OPERATING CONDITIONS

ICP torch	DT-B20 (Cetac Technologies)
Argon outer flow-rates	17 l/min
Argon intermediate flow-rate	0.7 l/min
Nebulizer gas pressure	550 kPa (80 p.s.i.)
Plasma forward power	1.5 kW
Sample flow-rate	80 μl/min
Integration time	3.0 s

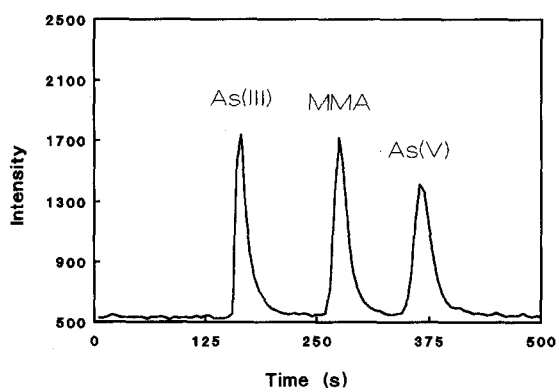

Fig. 2. Anion-exchange separation of arsenic species. Column ANX1710 was used with a 5.0 m*M* NH_4HCO_3–$(NH_4)_2CO_3$ eluent. Eluent flow-rate was 80 μl/min with a 10-μl sample injection volume.

TABLE III

RESPONSE DATA FOR ARSENIC SPECIES

Species	Conventional Nebulizer –ICP-MS detection[a]	DIN–ICP-MS detection[b]	DIN-ICP-AES detection[c]
Arsenite	5.9	240	4170
Dimethylarsinate	11.2	220	
Monomethylarsonate	5.0	260	4740
Arsenate	2.4	230	4430

[a] Data in counts per picogram from ref. 18.
[b] Data in counts per picogram from ref. 12.
[c] Relative intensity for 10-μl injection, present work.

the species. The detection limit for arsenic is 10 μg/l, and the minimum detectable quantity is 100 pg.

Table III gives a comparison of detector response data for various forms of arsenic. The results in the first two columns were obtained with a conventional column with a pneumatic nebulizer and a microbore column with DIN–ICP-MS detection [12,18]. The last column shows data obtained with a microbore column and DIN–ICP-AES detection. Because different organoarsenic species have different volatilities, transport efficiency of the species into the plasma with conventional pneumatic nebulizers will vary. The DIN nebulizes all of the sample into the plasma, so the amount of a particular species entering the ICP is independent of its volatility. Therefore, introduction of all of the sample into the plasma by the DIN provides relatively uniform sensitivity for both ICP-AES and ICP-MS. Separate speciation experiments of Cr(III) and Cr(VI) and Se(IV) and Se(VI) also resulted in uniform AES sensitivity.

Besides direct calibration, another advantage of uniform response is the ability to mass balance the various species with the total elemental content of a sample. A sample may be separated into various elemental species. However, because of column selectivity differences, it is not known if all species eluted from the chromatographic column. The total elemental content of a sample can be determined by performing a second injection without the column present and keeping all other conditions constant. Elemental concentration without the column can be compared to the total concentration of the various species measured from the column separation.

Selenium

Selenium has been reported in the media for years. It has caused widespread poisoning of water fowl in several watershed areas of central California. Selenium has been washed or leached into these areas, mainly due to the widespread irrigation of selenium-containing farm soil. Selenite is roughly twice as toxic as selenate. At very low levels, selenium is recognized as an essential trace element in human and animal nutrition. In fact, vitamins often contain selenium.

Selenium has been speciated previously by anion-exchange chromatography [1,19–21]. The selenite and selenate speciation was achieved using identical column and eluent conditions as used in the arsenic speciation described above. A simultaneous separation of arsenic and selenium species is given in Fig. 3. Individual injections of the various oxidation

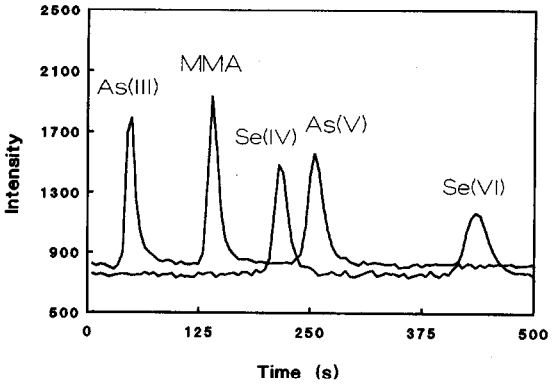

Fig. 3. Simultaneous separation of arsenic and selenium species. Column ANX1710 was used with a 5.0 m*M* NH_4HCO_3–$(NH_4)_2CO_3$ eluent. Eluent flow-rate was 80 μl/min with a 10-μl sample injection volume.

states of selenium and arsenic showed that no oxidation or reduction of the various species occurred in the sample mixture. The selenium detection limit is 4 μg/l (40 pg for a 10-μl injection).

Chromium

Cr(III) is an essential micronutrient. However, Cr(VI) is extremely toxic. Current methods for chromium speciation of environmental samples are lengthy and complex. Total chromium is determined by stabilizing a sample at pH 2 and then analyzing it by ICP-AES. Using US Environmental Protection Agency (EPA) Method 218.6 [22], Cr(VI) is determined by stabilizing a sample at pH 9, filtering it [which removes precipitated Cr(III)] and then using an anion-exchange ion chromatography method to measure chromate. The amount of Cr(III) in the sample is calculated by subtracting the Cr(VI) concentration from the total chromium concentration.

Our strategy for performing chromium speciation was to develop an anion-exchange method that could be carried out under acidic pH conditions so that both Cr(III) and Cr(VI) are soluble. Only a few methods with standard-bore anion-exchange columns have been reported [23,24]. Cr(III) is a cation and should travel unretained through an anion-exchange column. However, Cr(VI) is an anion and should be retained and eluted later. The major difficulty in this strategy is the possible precipitation of Cr(III) either prior to injection or during injection on the column. Because of this possibility a low-pH eluent was developed to prevent Cr(III) precipitation. No other sample preservation procedures were employed. Fig. 4 shows an unretained Cr(III) peak using a pH 2 eluent. A matrix up to 500 ppm sulfate with a 10-μl injection volume was tolerated without affecting the retention times. (In a similar study with arsenic and selenium, a background matrix of 500 ppm sulfate did have a small effect on the retention times and peak shapes. This is possibly due to the relatively low concentration of buffer eluent used in the arsenic–selenium studies.) The detection limit for chromium is 2 μg/l (20 pg for a 10 μl injection).

There is concern that low pH could convert Cr (VI) to Cr(III) [25]. In our experience, this was never observed. Experiments of individual species at a pH range of 2–9 showed that the lower pH keeps

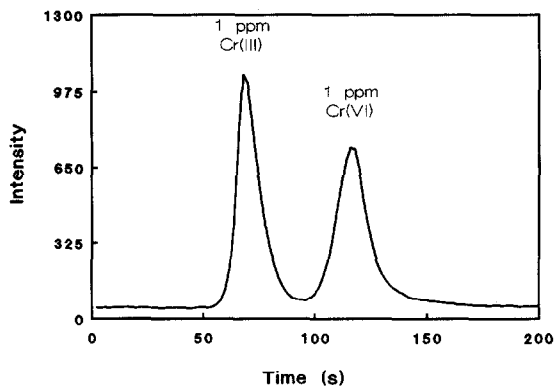

Fig. 4. Simultaneous separation of 1 mg/l each of Cr(III) and Cr(VI) in the presence of 500 mg/l of sulfate matrix. Column ANX1705 was used with a 110 m*M* NH_4NO_3 eluent (pH 2). Eluent flow-rate was 80 μl/min with a 10-μl sample injection volume.

Cr(III) dissolved, but does not facilitate conversion of Cr(VI) to Cr(III). The eluent was prepared by making up a solution of 110 m*M* nitric acid and adjusting the pH to 2 with ammonium hydroxide. Samples that are acidified give nicely shaped Cr(III) and Cr(VI) peaks. At pH 7, the Cr(VI) peak is well shaped; however, the Cr(III) peak is split. Even if the Cr(III) peak is poorly shaped, the concentration of Cr(III) will correspond to the peak area if analyzed promptly. Upon storage at pH 7, the Cr(III) precipitates over the course of a few days. If the sample is even more basic, Cr(III) is insoluble.

If a strong reducing agent is present under acidic conditions, Cr(VI) may be reduced to Cr(III) [25]. However, storage and stabilization of the sample at pH 9 is not acceptable because chromate will precipitate with cations such as lead. If EPA Method 218.6 is used, the sample is filtered, the pH adjusted to 9.0–9.5, and the sample refiltered. Partial or complete loss of Cr^{6+} may occur if Pb^{2+} or similar ions are present that form insoluble chromate salts under basic conditions.

The sample pH, not the eluent pH, may be the important factor with regard to conversion of Cr (VI) to Cr(III). In order to test this hypothesis, a number of reducing agents were added to Cr(VI) and the solution was analyzed for Cr(III) and Cr (VI). First a mixture of Se(IV) and As(III) was added to Cr(VI) at both pH 2 and pH 9. In neither case was Cr(III) generated. Next, excess Fe(II) was added to 1 mg/l of chromate and was found to reduce the Cr(VI) to Cr(III) over a period of 1 h.

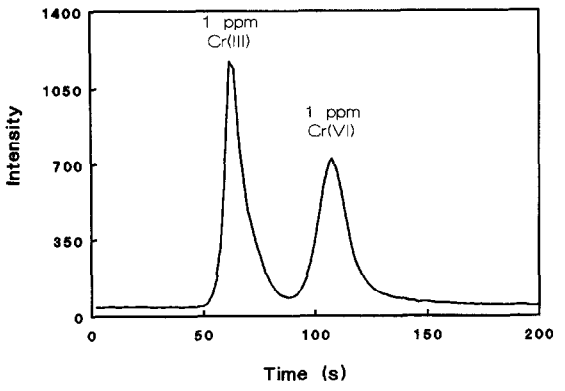

Fig. 5. Separation of 1 mg/l each of Cr(III) and Cr(VI) spiked into Omaha creek water. The unspiked sample did not contain detectable chromium. There was no oxidation or reduction of the chromium species in the creek water matrix. Column ANX1705 was used with a 110 m*M* NH_4NO_3 eluent (pH 2). Eluent flow-rate was 80 μl/min with a 10-μl sample injection volume.

Environmental water samples were spiked with 1.0 mg/l each of Cr(III) and Cr(VI). The samples were stabilized by mixing 50:50 with the acidic buffer eluent. A separation of the two chromium species spiked into Papio Creek water (Omaha, NE, USA) is given in Fig. 5. No species interconversion was noted over an eight-day period, indicating that these species are stable under acidic conditions.

CONCLUSIONS

Phase 1 of this work was to establish a family of columns that can interface with a DIN–ICP-AES detection scheme and separate a variety of elemental species. The separation conditions were chosen to operate with a variety of samples. The specific column, eluent, injection volume, etc. depend on the type and number of species to be separated, the sample matrix, sample stability and detection limits required. In general, conditions were chosen based on methods developed for standard columns so that published sample preparation procedures can be used.

Phase 2 of this work is to develop specific methods for particular samples. This is an ongoing process. Verification of the chromatographic conditions depends on making certain that the relative concentration of the particular species does not change during the course of sampling, sample preparation and analysis.

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