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Retention of Cr(III) by high-performance chelation ion chromatography interfaced to inductively-coupled plasma mass spectrometric detection with collision cell

Anne P. Vonderheide [a], Juris Meija [a], Katherine Tepperman [b], Alvaro Puga [c], Allan R. Pinhas [a], J. Christopher States [d], Joseph A. Caruso [a,*]

[a] *Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221-0172, USA*
[b] *Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221-0006, USA*
[c] *Department of Environmental Health, University of Cincinnati, Cincinnati, OH 45221-0056, USA*
[d] *Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY 40292, USA*

Abstract

High-performance chelation ion chromatography (HPCIC) was employed to retain cationic Cr(III) on an anion-exchange column and hence allow the separation of the two most prevalent forms of chromium, Cr(III) and Cr(VI). A mobile phase of nitric acid was utilized at pH = 1.5; additionally, 2,6-pyridinedicarboxylic acid was used at a concentration of 6 mM. Additives with different structural characteristics were used in an effort to elucidate retention mechanisms. Inductively-coupled plasma mass spectrometry was used for chromium detection. A collision cell was utilized to reduce chloride-based polyatomic ions that may interfere with the detection of Cr(III), and a detection limit study yielded levels in the low part-per-billion range. The newly developed method was applied to the chromatographic analysis of samples of an incubation medium containing Cr(VI) incubated with cell nuclei.
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1. Introduction

The two most prominent oxidation states of chromium that exist in nature are Cr(III) and Cr(VI); the intermediate states of $+2$, $+4$ and $+5$ are metastable and rarely encountered. At low pH, Cr(III) is most often found as a hexahydro cationic species, $Cr(H_2O)_6{}^{3+}$. As the pH is increased, water molecules are successively replaced with hydroxide ions until three ligand substitutions have occurred; at this point, precipitation occurs. Chromium(VI) usually exists as the chromate ion, $CrO_4{}^{2-}$ and may possess some degree of protonation depending on the pH. With both trivalent and hexavalent Cr, the species is pH dependent. By noting the cationic nature of Cr(III) and the anionic nature of Cr(VI) in an acidic medium, the difficulties in their chromatographic separation at lower pH become quite apparent.

Chromium enters the environment as a component of industrial wastes, such as that produced by the dyeing, tanning and steel industries. This may also occur as a result of the presence of Cr(VI) in artificial fertilizers. Chromium(III) is an essential element because it helps to activate insulin; on the other hand, chromium(VI), which is carcinogenic, has the capability of crossing cell membranes and then is reduced to Cr(III) in the cell [1–4]. Hence, there is an obvious need for measurement of the two species individually.

Several methods to separate the anionic and cationic forms of chromium have been published and a number of reviews summarize these methods [5,6]. Most use a complexing agent to impart a negative charge to the chromium(III) and subsequently use an anion-exchange column for separation. However, due to the fact that complexes of Cr(III), like those of Co(III), are kinetically inert to ligand substitution, the chelation procedures typically require high temperatures, although speciation analyses generally necessitate that samples be manipulated as little as possible to preserve the nature of the original species [7].

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* Corresponding author. Tel.: +1-5135-565858; fax: +1-5135-560142.
E-mail address: joseph.caruso@uc.edu (J.A. Caruso).

In this work, a method for the separation of Cr(III) and Cr(VI) in an acidic medium was developed that does not require complexation of Cr(III). It was the intention of the authors to use the method for the speciation of chromium in media exposed to nuclei in vitro to determine whether Cr(VI) was reduced during the incubation. For chromatographic separation of the two species, an anion-exchange column was employed in conjunction with the addition of 2,6-pyridinedicarboxylic acid. Detection was performed by inductively-coupled plasma mass spectrometry. This detector has been found to be both sensitive and selective and it additionally allows for time-resolved analysis and isotope profiling.

2. Experimental

2.1. Reagents and standards

All water was deionized (18 MΩ cm) and prepared by passing through a NanoPure treatment system (Barnstead, Boston, MA). Commercial chemicals were of analytical reagent grade and were used without further purification. Cr(III) standards were prepared from $CrCl_3 \cdot 6H_2O$ and Cr(VI) standards were prepared from $K_2Cr_2O_7$; both were obtained from Fisher Scientific (Fairlawn, NJ). Nitric acid, used for the mobile phase preparation, was also purchased from Fisher Scientific (Fairlawn, NJ).

H_2 and He, both with a purity of 99.999%, were used separately and exclusively as cell gases. The reactant gas flow was set and controlled by the mass flow controller provided with the instrument.

No interconversion between the Cr(III) and Cr(VI) species was observed in fresh solutions. Because of concerns of conversion of Cr(VI) to Cr(III) in acidic solutions, as well as oxidation of Cr(III) to Cr(VI) in air, standards in mobile phase were prepared fresh daily.

2.2. Instrumentation

2.2.1. HPLC conditions

The Agilent 1100 liquid chromatograph was equipped with the following: a binary HPLC pump, an autosampler, a vacuum degasser system, a thermostated column compartment and a diode array detector. The HPLC system was connected through a remote cable that allowed the simultaneous start of the chromatographic run on both instruments. An anion-exchange column (AS-11) (Dionex, Sunnyvale, CA) was used for separation. Packing particles were 13 μm in diameter; the surface anion-exchange latex layer was coated on an ethylvinylbenzene-divinylbenzene core. The column temperature was maintained at 25 °C for all experiments, although some have found that higher temperatures ensure that chelation is the dominant process with regard to ion exchange [8].

Table 1
Instrument operating conditions

ICP-MS parameters	
Forward power	1300 W
Plasma gas flow rate	15.0 l/min
Carrier gas flow rate	1.4 l/min
Sampling depth	6 mm
Sampling and skimmer cones	Nickel
Dwell time	0.1 s per isotope
Isotopes monitored	^{50}Cr, ^{52}Cr, ^{53}Cr and ^{54}Cr
Nebulizer	Meinhard
Spray chamber	Scott double-pass
Cell gas	H_2
Flow rate of cell gas	1.75 ml/min
HPLC parameters	
Column	Dionex AS-11 with dimensions of 250 mm × 2.0 mm and a particle size of 5 μm
Mobile phase	Nitric acid, pH = 1.5, 6 mM 2,6-pyridinedicarboxylic acid
Flow rate	0.1 ml/min at 0 min, 0.2 ml/min at 2 min, 0.5 ml/min at 3 min
Temperature	25 °C
Injection volume	25 μl

2.2.2. Inductively-coupled plasma mass spectrometry

An Agilent 7500c ICP-MS (Agilent Technologies, Tokyo, Japan) was employed for detection; this instrument is equipped with an octapole ion guide operated in an rf only mode. Instrument operating conditions are shown in Table 1. Chromium isotopes ^{50}Cr, ^{52}Cr, ^{53}Cr and ^{54}Cr were monitored. A platinum shield plate and bonnet, also know as Agilent's Shield Torch System, were used. This system is comprised of a grounded metal plate which lies between the plasma rf load coil and the torch and has the effect of removing the capacitive coupling between them.

2.3. Sample preparation

2.3.1. Nuclear sample preparation

Cells of the Hepa-1 cell line (mouse hepatoma) were plated at 2×10^6 cells/150 mm plate and incubated until 80% confluent in a medium containing Delbecco's modified minimal essential medium (MEM) and 5% fetal bovine serum, 1% antibiotic solution (10,000 U penicillin, 10 mg streptomycin/ml) (Invitrogen), at 37 °C in a 5% CO_2 atmosphere.

The medium was removed from the plates and the cells rinsed two times with ice-cold phosphate buffered saline (PBS). The PBS consisted of 2.7 mM KCl, 138 mM NaCl, 1.5 mM KH_2PO_4 and 8 mM Na_2HPO_4. Cells were removed from the plates using a cell lifter in 1 ml cold PBS, then centrifuged at 1500 rpm. The cell pellet was measured and 1 ml of lysis buffer (10 mM NaCl, 3 mM $MgCl_2$, 10 mM Tris, pH 7.5, 0.5% NP-40 (Nonidet P-40), prepared in DDI·H_2O, treated with DEPC (0.2 ml diethylpyrcarbonate/100 ml) and autoclaved) was added per two plates. The sample was mixed by pipetting and incubated on ice for 5 min. It was then centrifuged at 15,000 rpm in a microcentrifuge for 5 s. Nuclei

were resuspended in lysis buffer, counted with a hemocytometer, then centrifuged again. Nuclei were stored in nuclei storage buffer (40% glycerol, 50 mM Tris, pH 8.5, 5 mM MgCl$_2$), at 2×10^8 nuclei/ml.

2.3.2. Chromium exposure

A volume of 500 μl of nuclei was added to 500 μl of buffer containing 10 mM Tris, pH 8.0, 5 mM MgCl$_2$, 300 mM KCl. Nuclei were incubated in vitro with 100 μM K$_2$Cr$_2$O$_7$ for 30 min at room temperature. At the end of the incubation, nuclei were centrifuged as above. The supernatant was frozen rapidly on dry ice and thawed rapidly for speciation analysis.

3. Results and discussion

3.1. High-performance chelation ion chromatography

Several works report chromatographic separations of Cr(III) and Cr(VI) prior to detection. All must consider the impact of pH with regard to the complexes present in aqueous solution. Cr(III) exists as either Cr^{+3} or CrOH^{+2} at low pH but will precipitate as Cr(OH)$_3$ at pH higher than 5. On the other hand, at low chromium concentrations, hexavalent chromium exists primarily as HCrO$_4$$^-$ at low pH and then dissociates at pH > 7.

Retaining both the species in the column is important to avoid coelution with interfering ions in the void volume. To date, popular methods of separation consist of anion-exchange chromatography and ion-pairing chromatography. When employing anion-exchange chromatography, authors may use complexing agents to impart a negative charge to the Cr(III) complex. For example, Byrdy et al. chelated Cr(III) with EDTA in order to stabilize the trivalent ion in a basic mobile phase; solutions were heated at 50 °C for 1 h [9]. Tomlinson and Caruso complexed Cr(III) by heating in the mobile phase (containing 2,6-pyridinedicarboxylic acid and lithium hydroxide) at 65° for 2 h [10].

With ion-pairing chromatography, an organic modifier or salt is typically added to the mobile phase for quicker separations. Conventional cationic and anionic ion-pair reagents include alkylsulfonic acids and tetra-alkylammonium salts, respectively. However, solvents used in the mobile phase may destabilize the plasma. The increased formation of the ^{40}Ar^{12}C$^+$ diatomic ion in the presence of an organic mobile phase presents a further complication as the major isotope of Cr is $m/z = 52$. Finally, high salt concentrations are not recommended with ICP-MS detection because clogging of the torch tip and other orifices of the instrument may lead to decreased sensitivity as well as the possibility of space charge effects.

Others have employed alternate types of chromatography in the pursuit of Cr(III)/Cr(VI) separation. For example, Ali and Aboul-Enein [11] used reverse phase HPLC to separate the two chromium species; they utilized a C$_{18}$ column and a mobile phase of 90:10 acetonitrile:water, 10 mM ammonium acetate buffer (pH = 6.0). Andrle et al. also utilized a reverse phase HPLC column; they used ammonium pyrrolidinedithioate in the mobile phase to effect the formation of different complexes of Cr(III) and Cr(VI) [12]. Still other researchers have utilized capillary electorphoresis in the separation of the chromium species [13,14].

In general, speciation entails the coupling of a separation technique to a sensitive and selective means of detection. ICP-MS detection was chosen for its sensitivity and selectivity and hence, mobile phase constituents must be chosen accordingly. Others have used acidic mobile phases in conjunction with anion-exchange columns for the speciation of chromium [15,16]. In this work, a Dionex AS-11 column was employed for the retention of Cr(VI). This column has an anion-exchange layer that is functionalized with quaternary ammonium groups. Fig. 1a shows the separation results of Cr(III) and Cr(VI) with a mobile phase of 32 mM HNO$_3$ (pH = 1.5). Others observed the necessity of avoiding speciation of Cr(VI) at a pH < 1 due to the strong oxidizing properties of H$_2$CrO$_4$ [15]. As the pH is increased past the value of 2, water ligands of the Cr(III) species are successively replaced with hydroxide ions. Note Cr(III) elution in the void volume.

Because elemental species of interest may vary widely in chemical nature, chromatographic techniques that explore more than one separation mechanism are gaining popularity. High-performance chelation ion chromatography (HPCIC) is one such technique and it was explored as a means to effect retention of the cationic Cr(III) on an anion-exchange column. This type of separation takes advantage of both the retention characteristics of the column utilized, as well as the chelating capability of added complexing agents [17]. In other words, the acting separation mechanisms are both chelation and ion exchange; with chelation, separations are based on the formation and dissociation of metal complexes as well as electrostatic interactions. This technique has been documented to be quite useful in the chromatographic separation of metals in matrices of high ionic strength by including chelation as a separation mechanism in addition to ion exchange [18].

In practice, a chelating compound may be chemically bonded to the stationary phase substrate or may be added to the mobile phase [19], the latter known as "dynamic coating". In the second case, equilibrium is established between the amount absorbed on the chromatographic substrate and the concentration in the mobile phase [20]. Dynamic coating of the substrate is thought to occur through a combination of hydrophobic and π–π interactions between the aromatic group of the chelating agent and the benzene groups of the stationary phase support [21]. In this work, the dynamic mode of HPCIC was explored to effect the chromatographic retention of Cr(III) on an anion-exchange column that should inherently provide retention of Cr(VI).

A variety of complexing agents have been used with various stationary phases. In almost all papers to date, the
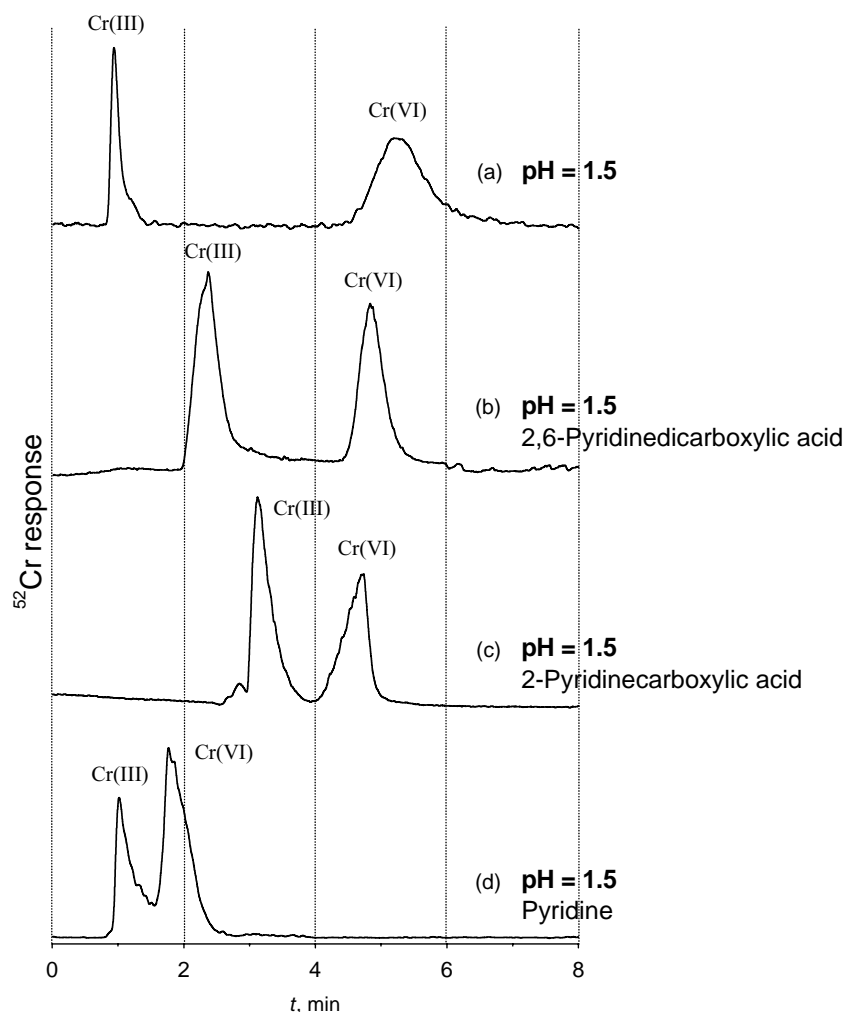
Fig. 1. Chromatograms of Cr(III) and Cr(VI) with a mobile phase of (a) HNO$_3$ at pH = 1.5, (b) HNO$_3$ at pH = 1.5 and 6 mM 2,6-pyridinedicarboxylic acid, (c) HNO$_3$ at pH = 1.5 and 6 mM picolinic acid and (d) HNO$_3$ at pH = 1.5 and 6 mM pyridine.

chelation agents have been found to add a unique selectivity to simple ion-exchange resins in that stationary phases modified with such are able to separate di- and trivalent metal cations. As stated earlier, the retention of metal ions on the chelating sorbent depends on both the complexation and electrostatic interactions of the metal cations with the chelating groups. It is known that Cr(III) has a high affinity for N, O and S electron donor atoms; work done by El-Shahawi [22] and other studies done by Maciejewska et al. [23] show that the carboxylate group of a sulfur amino acid participates in the formation of a Cr(III)–amino acid complex. Furthermore, the aminopolycarboxylate ligands have donor groups that are similar to these common amino acid residues [24]. With respect to HPCIC, Nesterenko et al. showed that when strong acid solutions with concentrations of 5×10^{-4} to 2×10^{-2} M are used as the eluants, the chromatographic stationary phases modified with iminodiacetic acid exhibited strong cation-exchange properties [25].

In this work, the use of 2,6-pyridinedicarboxylic acid was investigated in an attempt to effect retention of the Cr(III).

The pH value of the mobile phase is a crucial factor in determining the substituents available for chelation [26,27]. The pK_a values for 2,6-pyridinedicarboxylic acid are as follows: 0.46, 2.16 and 4.76; hence, the nitrogen of the ring will not be protonated and a small percentage of the molecules will contain a deprotonated carboxylate group at pH 1.5. In other work by Nesterenko et al., a N,O,O chelating system was found to yield bidentate O,O chelation at low pH [28].

Fig. 1b shows that the addition of 6 mM 2,6-pyridinedicarboxylic acid results in the retention of Cr(III). This concentration is slightly higher than typical modifier concentrations used by others. Many authors strive to keep the levels of chelating compounds in the mobile phase as low as possible as the additives may compete with analytes for post-column reagents or otherwise disrupt the detection scheme [29]. Since element-selective detection was employed here, this was not a concern. Furthermore, greater retention usually results with higher concentrations of chelating agents in the mobile phase [30]. Additionally,

the adsorption of the chelating agent on the resin of the chosen stationary phase is at a maximum at an eluant pH close to its isoelectric point. According to the pK_a values for the carboxylic groups and pyridyl nitrogen, the isoelectric point is near that of the established eluant pH [31].

In further experiments, a second chelating agent was investigated in an attempt to further elucidate the retentive mechanism governing the elution of Cr(III). Fig. 1c shows the use of another widely-used chelating agent in HPCIC. Picolinic acid (pyridinecarboxylic acid) differs from 2,6-pyridinedicarboxylic acid in that it has one less carboxylic acid group and its pK_a values are 1.07 and 5.25. Hence, as opposed to the 2,6-pyridinedicarboxylic acid agent, the nitrogen atom of picolinic acid will be partially protonated at the working pH of 1.5. Interestingly, the Cr(III) exhibits even greater retention with the use of this chelating agent and it elutes quite closely to the Cr(VI) species. Others have noted different chelating properties with picolinic and 2,6-pyridinedicarboxylic acid as well [29]. This leads to consideration of a greater role of the 2,6-pyridinedicarboxylic acid in the mobile phase, however, no experimental work supports this speculation. In an additional experiment, benzoic acid, differing from picolinic acid in the absence of the aromatic nitrogen, was used to determine the role, if any, of the nitrogen in the retention demonstrated. The resulting chromatogram showed (data not shown) the same retention as with picolinic acid, allowing the speculation that the nitrogen has no role in retaining the Cr(III), even to the point that its level of protonation is inconsequential.

To further confirm it is the oxygen atoms of the carboxylic acid moieties that are responsible for the change in retention, 2,6-pyridinedicarboxylic acid was replaced with pyridine. Because pyridine contains only the nitrogen of the ring structure and no carboxylate groups, the Cr(III) should exhibit no retention with this modifier. As can be viewed in Fig. 1d, this is found to be true. Additionally, the elution time of Cr(VI) is found to decrease substantially. The pK_a of pyridine is 5.2 and hence, at the pH of the mobile phase, all molecules are protonated and unhindered by neighboring substituents. It is therefore speculated that an ion-pairing mechanism may be responsible for the decreased elution time of the Cr(VI).

In a collective viewing of the chromatograms of Fig. 1, the use of 2,6-pyridinedicarboxylic acid was found to demonstrate the best retention and separation of the Cr species at the established pH. Interestingly, other authors have observed that the sorbed layer will eventually leach off when the eluent is changed to one containing no chelating agent [29] and this phenomenon was demonstrated in this laboratory. Over a period of four days, we were able to completely remove the 2,6-pyridinedicarboxylic acid and subsequently, any means of retention for the Chromium(III). Final separation parameters are given in Table 1. Note that a linear pressure gradient was employed to hasten the elution of Cr(VI).

3.2. Collision cell

Cr has four stable isotopes ^{50}Cr (4.35%), ^{52}Cr (83.8%), ^{53}Cr (9.50%), and ^{54}Cr (2.37%). Chromium is recognized as an element that is subject to interference by polyatomic ions formed in the plasma. The most common is $^{40}Ar^{12}C^+$ at $m/z = 52$ but others may include chlorine or nitrogen-based interferents such as $^{35}Cl^{16}OH^+$ or $^{37}Cl^{14}NH^+$. Researchers have explored many avenues in an attempt to remove these interferences in the determination of chromium. For instance, Pantsar-Kallio and Manninen utilized both a cation and an anion-exchange column in succession to separate nitrogen, chlorine, sulfur, and carbon-based interferences before detection with ICP-MS [32]. Others, employing similar chromatographic parameters, used a high-resolution ICP-MS instrument to specifically attenuate the chloride-based interference [16].

In the chromatography that had been established and optimized, consideration was given to the robustness of the method with regard to particular samples that may enable the formation of interfering ions. Specifically, in the analysis of samples containing a large chloride concentration, study of the isotopic ratios in the Cr(III) peak showed that $m/z = 52$ and 54 were disproportionately high and this led to speculation regarding the formation of $^{35}Cl^{16}OH^+$ and $^{37}Cl^{16}OH^+$. Deionized water containing only Cl^- (through the addition of NH_4Cl) was analyzed and $m/z = 37$ was monitored. Results showed the elution of the chloride peak at the retention time of Cr(III). Fig. 2 shows the extent of interference of a chloride-based interference on $m/z = 52$.

A collision cell may be used to free specific isotopes of an analyte that may not be available [33]. This may serve to increase sensitivity if the specific isotope is most abundant or may allow a stronger conclusion with regard to the element present. This avenue was explored to eliminate this chloride-based interferent. A schematic and description of specific instrumentation is given elsewhere [34]. Additionally, a platinum shield plate and bonnet were employed, which reduces ion energy to <2 eV with a spread of <0.5 eV [35].

Collision/reaction cell ICP-MS utilizes a multi-pole ion guide contained in a cell and the cell is pressurized with a gas flow. Ions sampled from the plasma undergo an interaction with the gas prior to mass spectrometric analysis [36]. Conditions are adjusted to remove interferences while allowing the analyte to remain. Subsequently, it is essential to aim for the elimination of the isobaric interference while maintaining minimal scattering of the analyte ion. Removal of an interferent can be accomplished by collisional dissociation (collision energy must be higher than bond dissociation energy), reaction or energy discrimination. In this work, both hydrogen as a reaction gas and helium as a collision gas were investigated.

The background was monitored at $m/z = 52$ and 54 with the addition of increasing flow rates of both gases. (The cell pressure is represented indirectly by the flow rate.)
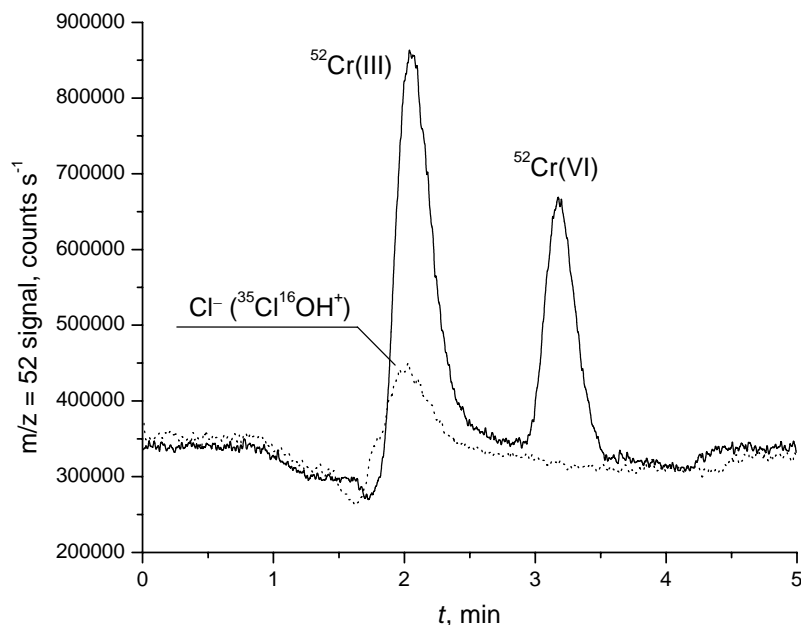
Fig. 2. Chromatogram of DDI solution of KCl overlaid with chromatogram of Cr(III) showing coelution of ^{52}Cr and ^{35}Cl^{16}OH ($m/z = 52$).

With the addition of helium, elimination of the interferent must be accomplished by either collisional dissociation or energy discrimination. In order for collisional dissociation to occur, the bond energy must be comparable to the collisional energy [37]. Energy discrimination relies on the fact that, for a given analyte and interference of equal mass and kinetic energy, the interferent will suffer more collisions due to its larger cross-section [38]. If ions all start with equal energy, collisions will selectively reduce the energy of interfering species. By imposing a stopping potential difference between the octapole and the quadrupole, interferent ions are rejected but analyte ions are allowed into the analyzer [39]. In this work, varying the flow rate of

helium showed equivalent decreases in analyte and background signals as a result of scattering losses. However, the desired elimination of the isobaric interference while maintaining the kinetic energy of the analyte ion was not observed.

Hydrogen is regarded as a reaction gas when introduced into the collision cell. In the examination of the plot of background signal versus hydrogen flow rate in Fig. 3, one can observe a more pronounced decrease in the background signal relative to the analyte signal. The value of background equivalent counts (BEC) is calculated as the ratio of the count rate of the background to the background corrected signal of a standard and is used to as a measure of optimal
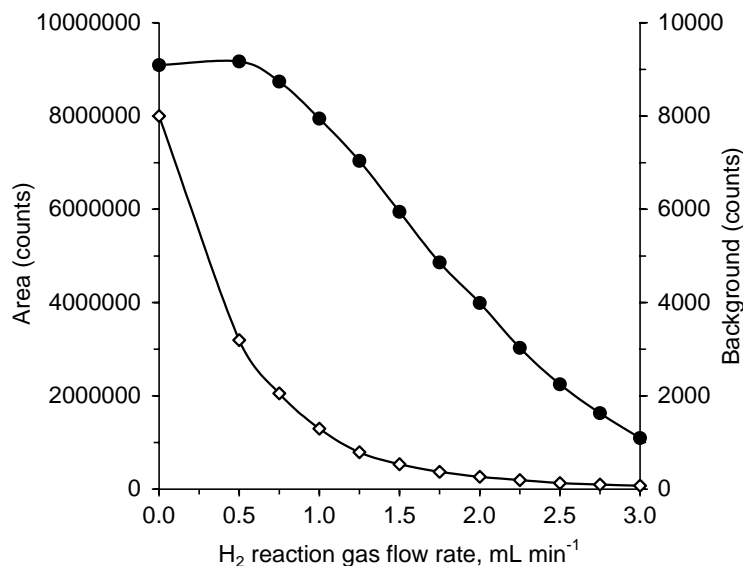

Fig. 3. Optimization of hydrogen as reaction/collision gas. (●) Peak area for chromium, (◇) background counts.

flow rate. For this work, a hydrogen flow rate of 1.75 ml/min was found to yield an acceptable BEC of 0.12 μg/l.

There are two possible results of using hydrogen as a reaction gas. First, there is the potential for an ion–molecule interaction that might result in the addition of a hydrogen atom to an ion, whether interferent or analyte. This would result in the formation of the unstable $ClOH_2^+$ and subsequent loss of water. Second, it may also happen that the charge of the isobaric interferent may be transferred to a hydrogen ion. However, the exact mechanism for the interferent removal cannot be truly understood with the instrumentation employed.

In Fig. 4, one can observe the intensity of $m/z = 54$ was diminished in the amount to be expected if it did indeed represent a ^{37}Cl-based interferent. However, it was still highly abundant in light of the expected chromium isotopic pattern and this is attributed to the ubiquitous presence of
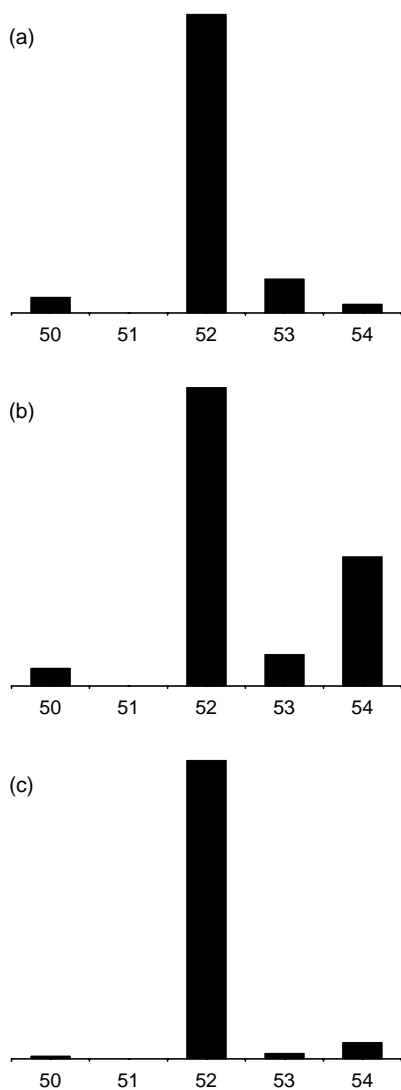

Fig. 4. (a) Chromium isotope pattern, (b) mass spectra of Cr(III) with H_2 reagent gas and (c) mass spectra of Cr(III) without H_2 reagent gas.

Table 2
Figures of merit

	Cr(III)	Cr(VI)
Regression coefficient	0.9997	0.9999
LOD, concentration (ppb)	0.3	0.8
LOD, amount (pg)	7.5	20
R.S.D., retention time, $n = 10$ (%)	2.4	2.4

iron. Consequently, this chromium isotope may not be used quantitatively or qualitatively.

3.3. Forward power

The incident rf power of the plasma is another common parameter that is optimized when utilizing ICP-MS. The results obtained by the application of various forward powers in conjunction with previously described chromatographic parameters show 1300 W as most favorable.

3.4. Chromatographic figures of merit

The method blank for the sample extraction set showed no detectable chromium species. Calibration curves were prepared with standards that ranged from 10 to 250 ppb. All regression coefficients were acceptable, with the lowest value being 0.9997. Specific figures of merit are shown in Table 2 and were collectively derived from the total ion current of the three chromium isotopes.

3.5. Instrument detection limits

The method used is based on sideband noise and utilized the following formula [40,41].

$$DL = \lim_{\phi_i \to DL} \left\{ \phi_i \frac{3s_b}{A_i} \sqrt{n} \right\}$$

The variable, ϕ_i, represents the volume fraction of analyte (ppb), s_b is the average standard deviation of the background noise near the peak of the analyte, A_i is the peak area of the analyte and n is the number of data points in the analyte peak. Detection limits were calculated from three of the calibration standards (10, 50 and 250 ppb). A detection limit of 0.3 ppb was calculated for Cr(III) and of 0.8 ppb for Cr(VI). Regardless of the level of the standard utilized in the calculation, the resulting detection limits are fairly constant and this is a consequence of the inverse relationship of the volume fraction to peak area. Finally, the limits of detection compare favorably with those of other Cr speciation methods [15].

3.6. Sample analysis

Samples were prepared as described in Section 2 and were kept frozen until analysis. Nuclei were prepared from Hepa-1 cells and exposed in vitro to 100 μM $K_2Cr_2O_7$ for
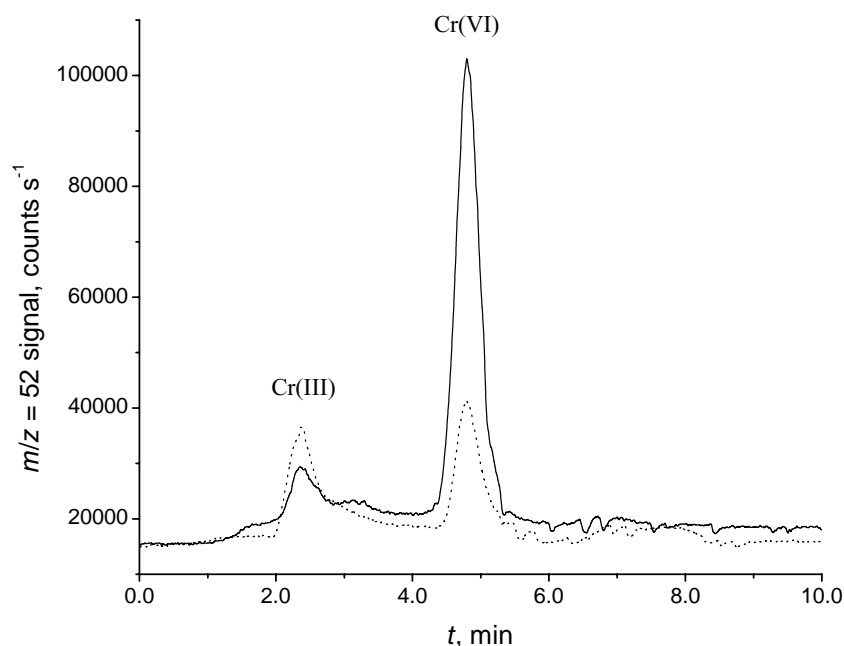
Fig. 5. Chromatogram of 50 ppb Cr(III) and 50 ppb Cr(VI) standard ($\cdots$) and medium sample (—). Nuclei were prepared from Hepa-1 cells and exposed in vitro to 100 μM $K_2Cr_2O_7$ for 30 min at room temperature. The medium was subjected to chromatographic separation after the nuclei were removed by centrifugation. The medium was diluted 1:10 before analysis.

30 min at room temperature. The medium was subjected to chromatographic separation after the nuclei were removed by centrifugation. As is shown in Fig. 5, in a 30 min exposure to nuclei in vitro, approximately 10% of the Cr(VI) is reduced to Cr(III).

4. Conclusions

High-performance chelation ion chromatography was successfully employed to retain cationic Cr(III) on an anion-exchange column. An acidic mobile phase at pH = 1.5 was employed in conjunction with the addition of 2,6-pyridinedicarboxylic acid at a concentration of 6 mM. The retention and separation of the two species was obtained in approximately 6 min. Detection was performed by monitoring chromium's three most abundant isotopes at $m/z = 50$, 52, and 53. A collision cell was employed to remove potential chloride-based polyatomic ions that would otherwise interfere with the detection of Cr(III). Hydrogen was used at a flow rate of 1.75 ml/min, however, chromium's least abundant isotope, ^{54}Cr, remained unavailable due to the ubiquitous presence of iron at mass 54. The new method was successfully applied to the analysis of nuclei samples incubated with Cr(VI).

Acknowledgements

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