Chromium speciation using an automated liquid handling system with inductively coupled plasma — mass spectrometric detection

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Abstract

The speciation of inorganic chromium in environmental samples is required for accurate assessment of pollution levels. Of the two chromium oxidation states, Cr (VI) is a known carcinogen, while Cr (III) is an essential element. Total chromium measurement cannot be used to determine actual environmental impact due to the considerable difference in toxicity of the two elemental forms. An automated liquid handling system, the PrepLab™, can be used with an inductively coupled plasma-mass spectrometer (ICP-MS) to quantify Cr (III) and Cr (VI) in liquid samples. An autosampler is used to introduce discrete sample volumes into a solid-phase chelation resin column. The Cr (III) and Cr (VI) species are separated and are introduced on-line into the VG PlasmaQuad 3 ICP-MS for detection. The chromatographic data are collected in time resolved analysis mode with the capability of simultaneous multiple-isotopic detection. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Chromium is a naturally occurring element found in rocks, animals, plants, soil, and in volcanic dust and gases. Humans are exposed when eating food, drinking water, and inhaling air that may contain chromium. The average daily intake from air, water, and food is estimated to be 0.01–0.03 µg, 2 µg/l, and 60 µg, respectively.

Dermal exposure to chromium may occur during the use of products that contain chromium such as wood treated with copper dichromate or leather tanned with chromic sulfate. Occupational exposure to chromium occurs from chromate production, stainless-steel production, chrome plating, and the tanning industries. Occupational exposure can be two orders of magnitude higher than exposure in the general population. People who live in the vicinity of chromium waste disposal sites or chromium manufacturing and processing plants have a greater probability of elevated chromium exposure than the general population.
Chromium occurs in the environment in two major valence states, trivalent chromium (Cr (III)) and hexavalent chromium (Cr (VI)). Cr (III) compounds are sparingly soluble in water, while Cr (VI) compounds are readily soluble which may result in enhanced levels of Cr (VI) in water sources. Cr (VI) is more toxic than Cr (III) and is a known carcinogen. The reference dose (RfD)\(^1\) for Cr (VI) is 0.005 mg/kg per day and the RfD for Cr (III) is 1 mg/kg per day. The US Environmental Protection Agency (EPA) estimates that consumption of these doses or less over a lifetime is unlikely to result in the occurrence of chronic non-cancer effects. Cr (III) is also an essential element in humans, with a daily recommended intake of 50–200 µg/day for an adult; it is essential to normal glucose, protein, and fat metabolism. The body is capable of detoxifying some Cr (VI) to Cr (III) which leads to increased levels of Cr (III) in the body. Laboratory tests can detect chromium in the blood, urine, and hair of exposed individuals, however it is difficult to separate Cr (VI) from Cr (III); therefore analysis is usually limited to total chromium.

1.1. Chromium speciation

Due to the widely different toxicity of the two forms of chromium, total chromium measurement cannot be used to determine actual environmental impact. The speciation of chromium in environmental samples is therefore necessary to accurately assess pollution levels. EPA Method 7196A was developed for the determination of Cr (VI) in EP/TCLP extracts and ground waters [1]. The method requires the addition of diphenylcarbazide and sulfuric acid to the water sample followed by measurement of the solution absorbance at 540 nm. A method using ion chromatography (IC) to separate Cr (VI) from Cr (III) prior to complexation with 1,5-diphenylcarbohydrazide and absorbance measurement has also been reported by the EPA [2]. This IC method utilizes an additional ICP-MS measurement to compensate for the reduction of Cr (VI) to Cr (III) during sample collection. Without the use of the ICP-MS data, the Cr (VI) measurements would be erroneous.

Chromium speciation methods utilizing chromatographic column separations followed by spectrometric detection also have been reported. Cox and coworkers used an activated alumina micro-column in an FIA manifold to separate and pre-concentrate Cr (VI) from Cr (III) followed by inductively coupled plasma atomic emission spectrometric detection [3]. A method using a chelating ion-exchange column to separate and pre-concentrate Cr (III) from Cr (VI) followed by flame atomic absorption spectrometric detection has been reported by Milosavljevic and coworkers [4].

Recent definitive studies of the interconversion of Cr (III) to Cr (VI) and Cr (VI) to Cr (III) during sample preparation call to question the accuracy of results generated using previously reported analytical procedures [5,6]. Speciated isotope dilution mass spectrometry (SIDMS)\(^2\) can be used to study and correct for these interconversion reactions during sample analysis [7].

\(^1\)The RfD is not a direct estimator of risk but rather a reference point to gauge the potential effects. Exceeding the RfD does not imply that an adverse health effect would necessarily occur. As the amount and frequency of exposures exceeding the RfD increase, the probability of adverse health effects also increases. The RfD for Cr (VI) is 0.005 mg/kg per day of body weight based on no effects noted in rats exposed to chromium in the drinking water. The RfD for Cr (III) is 1 mg/kg per day based on no effects observed in rats exposed to Cr (III) in the diet.

\(^2\)Speciated Isotope Dilution Mass Spectrometry (SIDMS). Isotope Dilution Mass Spectrometry is a highly accurate technique that has been used for the determination of metals in a range of matrices. The methodology relies on addition of an internal spike of the same element, of known but different isotopic composition to that of the sample for calibration. Also any loss of metal during sample preparation is compensated for, since the only measurement made is for isotopic ratios. SIDMS takes the method one stage further by addition of species-specific isotopically-enriched spikes, one spike for each metal specie to be measured. Therefore when the isotope ratios are determined using ICP-MS after chromatographic separation it is possible to calculate the original concentration of each species in the original sample. Obviously great care should be taken to avoid loss of speciation during sample preparation and analysis in order that the isotopic ratios can be determined accurately and precisely.
1.2. Chromium detection

Many spectrophotometric techniques are available for the detection of chromium, e.g. UV-VIS Spectrophotometers, atomic absorption spectrometers (AAS), and inductively coupled plasma spectrometers (ICP) all of which have different detection limit capabilities. Inductively coupled plasma–mass spectrometry (ICP-MS) has the advantage of achieving very low ng/l detection limits for chromium, which are needed for the analysis of environmental samples. ICP-MS is also relatively easily interfaced to many liquid chromatographic techniques [8] to allow automated, on-line separation and detection of the chromium species at ng/l concentrations in solution. Lastly, ICP-MS provides multiple-mass isotopic data for the separated species.

In this work an automated method was developed for the separation and determination of Cr (VI) and Cr (III) using low-pressure chromatographic separation of the species coupled with on-line ICP-MS detection. The method was developed to demonstrate analytical capability before implementation of a SIDMS procedure for chromium speciation in environmental samples.

2. Experimental section

2.1. Reagents and materials

All solutions were prepared using 18 MΩ deionized water. The following chemicals were used for preparation of sample loading solutions and eluting solutions: ammonium acetate (A.C.S. grade, Fisher Scientific, Pittsburgh, PA, USA), ammonium sulfate (A.C.S. grade, Fisher Scientific, Pittsburgh, PA, USA), ammonium nitrate (A.C.A. grade, GFS Chemicals, Columbus, OH, USA), ammonium hydroxide (cleanroom grade, Ashland Chemicals, Columbus, OH, USA), and nitric acid (redistilled, GFS Chemicals, Columbus, OH, USA). Individual certified stock solutions containing 1000 ppm Cr (III) and Cr (VI) (High Purity Standards, Charleston, South Carolina, USA) were used to prepare test solutions of the two chromium species.

2.2. Ion chromatography

Chromatographic separations of the chromium species were achieved using the PrepLab™; a metal-free, automated, liquid handling system (VG Elemental, Winsford, UK) (Fig. 1). The output of the PrepLab was connected directly by a short length of polyethylene tubing to the sample introduction system of the ICP-MS. Two, solid-phase chelation resins were evaluated: Chelex 100 (50–100 mesh, Bio-Rad Laboratories, Hercules, CA, USA) and Prosep-Chelating I (particle size 75–125 μm, Bioprocessing Limited, Consett, Co Durham, UK). The chelation resins were hand-packed into 3 mm × 25 mm glass columns with standard ½”-28 tube fittings and PTFE frits (OmniFit, Cambridge, UK). An Oakton pH Tester 3 (Cole-Parmer, Vernon Hills, IL, USA) was used to measure the pH of the mobile phases and solutions.

2.3. Inductively coupled plasma–mass spectrometry

A PlasmaQuad 3TM ICP-MS instrument (VG Elemental, Winsford, UK) fitted with an AutoR-range PlusTM simultaneous detector and 64 000 channel Multi-channel Analyzer was used for chromium detection. The ICP-MS sample introduction system consisted of a concentric nebulizer (Glass Expansion, Victoria, Australia) and a low-volume impact-bead quartz spray chamber. A low volume, sample introduction system with rapid
Metals are reversibly bound to the functional groups depending on the solvent in contact with the co-polymer. Chelex 100 has been used routinely to remove metal contamination from various solvents and to pre-concentrate metals for analysis \[9,10\]. The resin functions as a chelating resin from pH 4 to 12 and as an anion exchanger below pH 4.

MilliQ water, 0.05 M ammonium hydroxide, and 10 mM ammonium sulfate adjusted to pH 9 with ammonium hydroxide were evaluated as loading solutions for the separation of Cr (III) and Cr (VI). The eluting solution for all tests was 2 M nitric acid.

Since ICP-MS cannot differentiate between Cr (III) and Cr (VI) the binding specificity of the resin for each species was evaluated independently. Individual solutions containing 50 ppb Cr (III) and 50 ppb Cr (VI) in deionized water were prepared. Neither species exhibited specificity for the Chelex 100 resin with any of the three loading solutions.

A solution of 0.4% nitric acid was also tried as the loading solution since, at low pH, the resin behaves as an anion exchanger. A slight increase in the Cr signal followed by a leveling off was observed during the first 100 s after the Cr (VI) was introduced to the column. A wash of 2 M nitric acid did not produce any additional signal. The Cr (VI) appeared to initially bind to the resin and then slowly elute from the column with time. A much stronger binding between a Cr species and the resin is required for this separation to be effective.

3.2. Evaluation of Prosep-Chelating I

Prosep-Chelating I contains the same iminodiacetic acid functional group as Chelex 100 but the stationary phase support is porous glass rather than a co-polymer. Prosep-Chelation I functions as a chelating resin in the pH range from 1 to 9 and has been used to pre-concentrate metals for analysis (S. Nelms, 1997, personal communication). A 50 mM ammonium nitrate solution adjusted to pH 9 with ammonium hydroxide was evaluated as a loading solution. In this study 1 M nitric acid was used as the eluting solution.
Individual solutions of 50 ppb Cr (III) and 50 ppb Cr (VI) in water were analyzed first to evaluate the binding specificity of the resin. During sample loading with the 50 mM ammonium nitrate solution, Cr (VI) was not bound and flowed through the column to the ICP-MS detector. On the other hand, Cr (III) remained bound to the resin during sample loading with the 50 mM ammonium nitrate solution. When the eluent was switched to 1 M nitric acid, the bound Cr (III) eluted from the column. A solution containing both Cr (III) and Cr (VI) was analyzed next in order to test the separation capabilities of the resin (Fig. 2). Prosep-Chelating I demonstrated the required specificity for the separation of the two Cr species, which were separated in less than 5 min.

In order to further test the resin, individual solutions of Cr (III) and Cr (VI) in water were analyzed using the same analytical conditions. Fig. 3 illustrates that while Cr (III) resulted in only one peak at the Cr (III) retention time, Cr (VI) analysis resulted in peaks with retention times of both Cr (III) and Cr (VI). The small amount of Cr (III) detected during the analysis of the Cr (VI) solution could be caused by either Cr (III) contamination in the Cr (VI) solution or inter-conversion of Cr (VI) to Cr (III) during the analysis.

4. Discussion

To investigate whether the small amount of Cr (III) detected during analysis of the Cr (VI) solution was caused by contamination in the certified solution, further information on the production of the solution was obtained from the manufacturer. The manufacturer does not perform a Cr (VI)-specific test on the solution to obtain the certified concentration, but instead assumes that the solution contains only Cr (VI) because it is prepared from 99.998% pure solid potassium dichromate in water. Upon testing the pH of the standard solution it was determined to be acidic (pH 3.5). At pH less than 6.5, the predominant Cr (VI) species is the strong oxidizer hydrogen chromate. Under these acidic conditions, Cr (VI) can be converted to Cr (III) [2] and thus create Cr (III) contamination in the certified Cr (VI) solution. On the other hand, the basic conditions (pH 9.0) used during steps 1 and 2 of the IC-ICP-MS method described in this paper would discourage the conversion of Cr (VI) to Cr (III). Further studies using SIDMS at each step of the analysis procedure are planned in order to determine definitively the source of the Cr (III) contamination. Analysis of the Cr (VI) standard using a method such as EPA Method 7196A would not provide a definitive answer to the question of the source of the Cr (III) contamination as noted earlier.

Fig. 2. Chromatography of a mixture of Cr (III) from Cr (VI) using Prosep-Chelating I.
5. Summary

Cr (III) and Cr (VI) can be successfully separated using Prosep-Chelating I. On-line ICP-MS detection is sensitive enough to meet US EPA criteria. Further studies are necessary, however, to complete the evaluation of this method for the analyses of real environmental samples. Additional studies of interconversion of Cr (III) and Cr (VI) must be performed using SIDMS as well as investigation of possible interferences due to matrix ions such as Fe, Ca, K, and Na, which are present in water samples. Evaluation of sample storage containers and preservatives is also needed so that the chromium speciation in the sample remains unchanged from sampling site to analysis. Lastly, an evaluation of column design is planned to study peak shape and peak broadening.

References