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Occurrence and Speciation of Polymeric Chromium(III), Monomeric Chromium(III) and Chromium(VI) in Environmental Samples

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Abstract

Laboratory experiments suggest that polymeric Cr(III) could exist in aqueous solution for a relative long period of time. However, the occurrence of polymeric Cr(III) has not been reported in environmental media due partially to the lack of method for speciating polymeric Cr. We observed an unknown Cr species during the course of study on speciation of Cr in the leachates of chromated-copper-arsenate (CCA)-treated wood. Efforts were made to identify structure of the unknown Cr species. Considering the forms of Cr existed in the CCA-treated woods, we mainly focused our efforts to determine if the unknown species were polymeric Cr(III), complex of Cr/As or complex of Cr with dissolved organic matter (DOM). In order to evaluate whether polymeric Cr(III) largely exist in wood leachates, high performance liquid chromatography coupled with inductively coupled mass spectrometry (HPLC-ICPMS was used) for simultaneous speciation of monomeric Cr(III), polymeric Cr(III), and Cr(VI). In addition to wood leachates where polymeric Cr (III) ranged from 39.1 to 67.4 %, occurrence of the unknown Cr species in other environmental matrices, including surface waters, tap and waste waters, was also investigated. It was found that polymeric Cr(III) could exist in environmental samples containing $\mu\text{g/L}$ level of Cr, at a level up to 60% of total Cr, suggesting that polymeric Cr(III) could significantly exist in natural environments. Failure in quantifying polymeric Cr(III) would lead to the underestimation of total Cr and bias in Cr speciation. The environmental implication of the presence of polymeric Cr(III) species in the environment deserves further study.

Keywords

Chromium speciation; Cr(III); polymeric Cr(III); Cr(VI); CCA

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INTRODUCTION

Chromium (Cr), primarily existing as mineral chromite in Earth crust, is introduced into the environment through various industrial processes, such as metallurgic, wood preserving, tanning and plating industry (Metze et al., 2005; Hingston, et al., 2001). It is an element with multiple oxidation states with trivalent [Cr(III)] and hexavalent [Cr(VI)] being the two primary thermodynamically stable oxidation states in natural environment. Previous studies suggested that these two forms of Cr possess distinct environmental behavior and toxicity to human (Stewart et al., 2003; Metze et al., 2005; Santonen et al., 2009). Cr(VI) is known to be carcinogenic and mutagenic to human, and also one of the most commonly encountered occupational hazards (IARC, 1990). On the other hand, Cr(III) is generally considered as an essential micronutrient for human associated with glucose metabolism. Cr(III)-based nutritional supplements are widely used as human mineral supplements, particularly marketed for weight loss and performance enhancement. However, consensus on the beneficial role of Cr(III) no longer exists and this practice has been questioned because of the low efficacy and potential toxicity of such supplements (Levina and Lay, 2008). Moreover, Cr(III) is found to be more reactive than Cr(VI) toward DNA under *in vitro* conditions, and its genotoxicity to bacterial cells have also been suggested (Qi et al., 2000; Plaper et al., 2002). Although Cr(III) biochemistry in molecular level still remains unclear, it has been reported that only polymeric form may trigger Cr function on mediating of glucose metabolism, in which the Cr assembly contains four Cr(III) ion with a unique type of multinuclear assembly (Davis et al., 1997; Vincent, 2000; Jacquamet et al., 2003). These studies suggest that polymeric Cr(III) could play an important role in the biological function of Cr(III).

In aqueous solution, Cr(III) could form strong complexes with hydroxides and the species is subject to change depending on pH value. It mainly exists as $\text{Cr}(\text{OH})^{2+}$ at pH values from 3.8 to 6.3, and tends to form polymeric Cr(III) (Stumm and Morgan, 1996; Rai et al., 1989). As an intermediate step during the slow transition from free ions to precipitates, the formation of polymeric Cr(III) in water solution under the condition of saturation has been well documented. These polymeric Cr(III) species could count for up to 25% of the total Cr in solution and be stable in these metastable forms for years (Stunzi and Marty, 1983; Spiccia and Marty, 1986; Stunzi et al., 1989; Saleh et al., 1996). Different oligomers of the polymeric Cr(III) have various reactivity during the oxidation to Cr(VI) (Rao et al, 2002). However, the polymeric species of Cr(III) in environmental samples have been seldom considered due in part to the lack of a convenient method for simultaneous detection of monomeric Cr(III), polymeric Cr(III) and Cr(VI). The analytical method recommended by USEPA considers only Cr(VI) and total Cr (US-EPA, 1986b, a, 1992, 1996), and Cr(III) is calculated by the difference of total Cr and Cr(VI) (Song et al., 2006; Saputro et al., 2014), causing potential bias in estimating the environmental behavior and toxicity of Cr. Other methods either coped with a single Cr species (Aydin and Soylak, 2010; Elci et al., 2010) or utilized the similar strategy as the USEPA's method (Bulut et al., 2009). The quantification of both Cr(III) and Cr(VI) simultaneously remains a challenge because the high reactivity of Cr(III) and the presence of interfering ions and substances, such as, carbonate, chloride, and humic acid, which could dramatically interfere with the chromatographic separation of

Cr(III) (Seby et al., 2003). Complimentary methods have been tested to cope with the difficulties in speciation of Cr(VI) and Cr(III) in pore water samples contaminated by tannery effluent (Burbridge et al., 2012).

Chromated-copper-arsenate (CCA)-treated wood was widely used in the construction of outdoor structures in the United States since 1970s'. Metal/metalloids including Cr in the treated woods are leached at levels potentially toxic to human and other organisms (Hasan et al., 2010; Santonen et al., 2009). Wood treatment industry voluntarily withdrew the treated products for most residential settings since January 1, 2004. However, health problems associated with exposure to the metals from CCA-treated wood are still a topic of interest worldwide. This is because the utilization of CCA-treated wood in various fields, including industrial applications, structures in marine environments, and load bearing components of structures in terrestrial environments continues (Hu et al., 2010) and metal release will likely continue for at least several decades due to its long service life for the CCA-treated wood sold for residential and industrial uses prior to 2004 (Khan et al., 2006).

In our study on speciation of Cr in the leachates from CCA-treated wood using high performance liquid chromatography-induced coupled plasma mass spectrometry (HPLC-ICPMS), an unknown Cr species counting for a large portion of total Cr (~ 39 – 67%) was observed and found to be neither monomeric Cr(III) nor Cr(VI). Such an unknown Cr species seems to be observed at $\mu\text{g L}^{-1}$ levels in other previous studies on Cr speciation. Unfortunately, it was not identified or discussed (Wolf et al., 2007). It was also reported that the formation of unknown Cr(III) species in the presence of hydrogen carbonate ions during chromatographic separation of Cr(III) and Cr(VI) (Seby et al., 2003). To better understand the Cr species leached from treated wood and its potential environmental impacts, it is necessary to identify the form of this unknown Cr species. Previous studies indicated that Cr(VI) present in the original formula is reduced to Cr(III) in the wood during the course of wood treatment (Bull, 2001; Song et al., 2006). Chromium exists mainly in two forms in the treated wood, As/Cr clusters and solid Cr(III) hydroxide. The As/Cr clusters consist of a Cr dimer bridged by an arsenate oxyanion, and the dimer serves to anchor the clusters to organic group in woody structure (Nico et al., 2004). The solid Cr(III) hydroxide include a large portion of polymeric species because large amounts of dimer, trimer, tetramer, hexamer, and highly condensed polynuclear species can be formed in the course of wood treatment (Bull, 2000). Therefore, it is possible that these polymeric species of Cr could consequently leached out by the rainfall and be found in the wood leachates. It was speculated that the unknown Cr species observed during the speciation of Cr in wood leachate using HPLC-ICPMS could be certain forms of polymeric Cr.

Wood leachate contains fairly high level of dissolved organic matter (DOM, dissolved organic carbon ranged from 11 to 59 mg L^{-1}) (Hu et al., 2013). It is also possible that the unknown Cr species were complexes of Cr (III) and DOM. These types of complexes have been reported and their stability has been studied in previous studies. It was suggested that most Cr(III) was bound to high molecular weight organic ligands in both natural waters and laboratory prepared humic substance solutions (Krajnc et al., 1995; Koshcheeva et al., 2007). Complexation with organic matter could alter the mobility of Cr(III) in the environment. Organic matter present in solid phase could retain Cr(III) in soil phase, while

complexation with DOM could keep Cr(III) in solution and increase its mobility (Kotas and Stasicka, 2000; Laborda et al., 2007). Previous studies have suggested the existence of Cr(III)-carboxylate binding in the complex of organic matter and Cr(III) (Fukushima et al., 1995). In addition, the toxicity of Cr(III) varies after complexation with organic ligands, and the toxicity differs with the type of complexing ligands (Shrivastava et al., 2005; Levina and Lay, 2008).

The purpose of this study was to identify the unknown Cr species present in the CCA-treated wood leachates. Considering the forms of Cr existed in the CCA-treated woods, we mainly focused our efforts to determine if the unknown species were polymeric Cr(III), complex of Cr/As or complex of Cr/DOM. In order to quantitatively evaluate whether polymeric Cr(III) largely exist in wood leachates, a convenient method for simultaneous speciation of monomeric Cr(III), polymeric Cr(III), and Cr(VI) was developed. In addition to wood leachates, the occurrence of unknown Cr species in other environmental matrices, including surface waters, tap water and waste water, was also investigated.

EXPERIMENTAL

Chemicals and Materials

Cr(III) (1,000 mg L⁻¹) and Cr(VI) (1,000 mg L⁻¹) standard solutions were purchased from Ricca Chemicals Company. Ethylenediaminetetraacetic disodium dehydrate (EDTA, 99%) was purchased from Sigma. Tetrabutylammonium hydroxide (TBAH, 1.0 M solution in methanol) and methanol (100%, HPLC grade) were from Fisher Scientific. Deionized water was produced by a Barnstead water purification system. All other chemicals used were analytical or trace metal grade.

Instrumentation

HPLC-ICPMS (PerkinElmer, USA) was employed for Cr speciation. The system consisted of an ELAN DRC-e ICPMS coupled via two 6-port valves with a PerkinElmer serials 200 HPLC system. Post-column internal standard was pumped in and measured by ICPMS for each measurement, and the results were calculated with the correction of internal standard (Yehiayan et al., 2009; Hu et al., 2010). Oxygen was used as a reaction gas to eliminate the interferences for Cr analysis and ⁵²Cr was monitored for analysis of Cr. The HPLC-ICPMS system was operated with ChromeraTM software provide by PerkinElmer. The parameters used for the ICPMS and HPLC systems are listed in Table 1. Detail information on quantification analysis could be found in supporting information.

Electrospray ionization mass spectrometry (ESI-MS) analysis was performed on a Finnigan LCQ Deca XP MAX single quadrupole mass spectrometer (Thermo Electron Corporation, USA). For all spectral acquisitions, capillary voltage was set at 15 v, and source temperature was kept at 300 K. Samples were injected in direct infusion mode with an optimum flow rate of 10 µL min⁻¹. Mass spectra were acquired by scanning from m/z 50 to 2000 for 1 min at positive ion mode. To enhanced ionization efficiency, samples were all mixed with methanol at the ratio of 1:1 before detection. DOC concentrations were measured by acidifying the water sample to pH < 2 with 3 mol L⁻¹ HCl, purging the sample with CO₂-free air and

analyzing for total carbon using a hot platinum catalyst direct injection analyzer (Shimadzu TOC 5000).

Sample preparation

Several water samples from various sources with different Cr concentrations were collected. CCA-treated wood leachates generated from rainfall were collected from a field leaching system set up at the University of Miami (Hasan et al., 2010). The system was utilized to evaluate the leachability of metals from CCA-treated woods when exposed to rainfall under natural conditions. The Cr concentration in the leachate ranged from less than 1.0 to around 5,000 $\mu\text{g/L}$. The pH value of the leachates were in the acidic to neutral range ($5.0 < \text{pH} < 7.0$). A waste water sample ($\text{pH} = 6.0$) was collected from a water treatment plant at a tannery factory in Ecuador. A wetland surface water sample ($\text{pH} = 6.5$) was collected from a canal near Florida International University (FIU). All samples were stored at 4 °C after collection, and filtrated with a 0.45 μm membrane prior to analysis. The leachates and wetland samples were analyzed within two days upon sample collection and the waste water samples were delivered via airmail and analyzed approximately one month after sampling.

Preparation of solutions containing polymeric Cr(III)

The solution containing polymeric and monomeric Cr(III) species was prepared according to a method published previously (Stunzi et al., 1989). Briefly, 10 mL Cr(III) solution (1,000 mg L^{-1} , in 1% HNO_3) were added into 8 mL NaOH solution (1.0 M). The pH value of the prepared solution was approximately 4.0 right after the two solutions were mixed. The solution was further diluted to a certain concentration according to the experimental purpose. The prepared solution was then aged for 140 days at ambient temperature, and aliquot samples were taken at different times for Cr speciation during this period of time. EDTA was added to stabilize monomeric Cr(III) by forming complex of Cr(III)-EDTA during sample preparation and measurement. The stability of Cr(III)-EDTA was therefore evaluated. Briefly, the solution of Cr(III)-EDTA with a concentration of 500 $\mu\text{g L}^{-1}$ was prepared by diluting the stock solution of Cr(III) with the mobile phase. The solution was aged for 3 month at ambient temperature, and aliquot samples were collected at different times for Cr speciation.

Examination of Cr(III) and DOM interaction

To examine whether the unknown Cr(III) could largely be the complex of Cr/DOM, Cr(III) or Cr(VI) in the concentration of 100 $\mu\text{g L}^{-1}$ was mixed with wood extract and then speciation was performed. The wood extract was prepared by extracting 1.0 g of the untreated wood sawdust (without CCA treatment) with 25 mL of DDI water for 24 h. The prepared wood extract contained 21.4 mg L^{-1} of DOC, similar to the average content of DOC in CCA-treated wood leachates.

RESULTS AND DISCUSSION

Polymeric species of Cr(III)

A solution of hydrolytic Cr(III) with a final concentration of 500 mg L^{-1} and pH of 4.0 was prepared according to a previously reported method (Stunzi and Marty, 1983; Stunzi et al.,

1989). Figure 1 shows the ESI-MS spectrum of Cr(III) solution aged for 140 days using a direct infusion technique. The peaks could be assigned to polymeric and monomeric Cr(III). The polymeric species could be assigned to $[\text{Cr}_{2n+1}\text{O}_{2n-1}(\text{OH})_{2n+3}(\text{H}_2\text{O})_{n+6} - (n-1)\text{H}_2\text{O}]^{2+}$ ($n = 1, 2, 3, \dots$). Monomeric species could be observed as well in the forms of $\text{Cr}(\text{OH})(\text{H}_2\text{O})_8^{2+}$, $\text{Cr}(\text{OH})(\text{H}_2\text{O})_{10}^{2+}$, $\text{Cr}(\text{OH})_2(\text{H}_2\text{O})_5^+$. The formation and characterization of polymeric Cr(III) species were demonstrated in previous studies via hydrolysis of Cr(III) in aqueous solution (Stunzi and Marty, 1983; Stunzi et al., 1989). Polymeric Cr(III), including dimer, trimer, tetramer, pentamer and hexamer could exist up to four years, allowing possible isolation by chromatographic techniques. The aged Cr(III) solution was then analyzed with HPLC-ICPMS and compared with freshly prepared Cr(III) and Cr(VI). A major peak appeared at retention time of 4.2 to 4.8 min, representing monomeric Cr(III) in the freshly prepared Cr(III) solution (Figure 2A). A group of new peaks emerged at retention time from 2.2 to 3.5 min in the aged Cr(III) solution compared with the freshly prepared one (Figure 2B). The emerged peaks were obviously not Cr(VI), the oxidation product of Cr(III) (Figure 2C). Combined with the ESI-MS results, it could be concluded that the emerged peaks shown in the chromatogram of the aged Cr(III) were most likely polymeric species of Cr(III). A small amount of the polymeric Cr(III) species could also be observed even in the freshly prepared solution in DDI water (Figure 2A), indicating the polymerization of Cr(III) is fast at the beginning stage. Through the long-term aging experiment, it was observed that the percentage of polymeric species increased over time, in agreement with the previously published results (Stunzi et al., 1989). The percentage of polymeric species accounted for ~ 5% of the total Cr(III) in the freshly prepared solutions and increased to ~ 25% after 140 d (Figure S2 & Figure S3). Moreover, it was observed that the pattern of polymeric species seems to change with the aging time, and a new peak at retention time of ~ 2.6 min was emerged. Previous study had noticed the unidentified Cr(III) forms in which an ion exchange column was used for speciation of Cr in solution. The unknown species of Cr(III) were proposed to be various hydrolytic complexes of monomeric Cr(III) occurring due to the changes in pH value. It was obviously not the case for our results since the pH was kept unchanged in our experiments. The new peak at ~ 2.6 min might be attributed to the presence of polymerized form of Cr(III), although further evidence is needed to support this hypothesis.

Quantification of monomeric Cr(III)/Cr(VI) and estimation of polymeric Cr(III) with HPLC-ICPMS

Ion- pair chromatography coupled with dynamic reaction cell ICPMS was previously used for simultaneous determination of Cr(III) and Cr(VI) (Neubauer et al., 2003; Wolf et al., 2007). This method was adapted and optimized for the analysis of monomeric Cr(III), polymeric Cr(III), and Cr(VI). EDTA prepared in HPLC mobile phase (5% methanol, 0.5 mM EDTA, 1.0 mM TBAH, pH = 7.2) was primarily employed for converting cationic aqua-hydroxo complex (mainly $\text{Cr}(\text{OH})_2^+$ at pH = 7.2) of Cr(III) to anionic complex $[\text{Cr}(\text{III})\text{-EDTA}]^-$, and then the anionic complex could be separated from anionic Cr(VI) (Neubauer et al., 2003; Wolf et al., 2007). Only one peak corresponding to monomeric Cr(III) was observed when Cr(III) in this solution was analyzed after stored at 4 °C for 3 months (Figure S4), indicating that the Cr(III)/EDTA ion pair could remain stable at least for 3 month. Given the stability of polymeric Cr(III) species and complex of Cr(III)/EDTA, it was

possible to conduct a simultaneous speciation of polymeric Cr(III), monomeric Cr(III) and Cr(VI). To avoid the transformation between polymeric and monomeric species during sample preparation, working stock solutions, calibration standards, and samples were all prepared or diluted with the mobile phase.

Concentrations of polymeric Cr(III) were estimated by comparing the signal with that of the monomeric Cr(III) since standard polymeric Cr(III) species were not available. This is reasonable considering ICPMS is an element specific detector. Nineteen aged Cr solutions were analyzed with HPLC-ICPMS, and total content of Cr in these samples was also quantified with ICPMS. For the results obtained with HPLC-ICPMS, the polymeric Cr(III) were estimated via calibration curve of monomeric Cr(III) and the total concentration was calculated by sum of monomeric Cr(III), polymeric Cr(III) and Cr(VI). Mass balance in these samples was then calculated by comparing the total Cr obtained using HPLC-ICPMS and that using ICPMS (Table 2). Mass balance of Cr in these samples ranged from 80.7 to 111.0%, indicating that it is feasible to estimate the concentration of polymeric Cr(III) with monomeric Cr(III) standard. Although percentage of polymeric Cr(III) in these samples varied between 5.6 and 21.5%, the mass balance did not show obvious correlation with the percentage of polymeric Cr(III) (Figure S5), further verifying the feasibility of this quantification method.

Presence of unknown Cr species in the CCA-treated wood leachates

A typical chromatogram of the wood leachate (pH 6.5) showed the presence of some unknown Cr species between 2.2 and 3.5 minute (Figure 2D), with similar retention times as the polymeric Cr(III). Given that Cr presents largely as polymeric Cr(III) in CCA-treated woods, it is most likely that the unknown Cr species in the wood leachates consist of at least partially the polymeric forms of Cr(III). As shown in Figure S6, the retention time of the unknown Cr species was distinct from the major As species – As(V), suggesting that the unknown Cr species did not contain As and was not a complex of Cr/As. Another possible Cr species that could exist was Cr(III)/DOM complexes because the high content of DOM (DOC ranged from 10 to 100 mg L⁻¹ with an average of ~20 mg L⁻¹) present in the wood leachates. When either Cr(III) or Cr(VI) (100 µg L⁻¹) was mixed with wood extract in the wood leachate control experiments, no additional Cr species were observed (Figure 3), demonstrating that the observed Cr species were not the complex of Cr/DOM.

Speciation of Cr in environmental samples

Previous studies on Cr speciation in environmental matrices mainly focused on Cr(III) and Cr(VI) (Gomez and Callao, 2006). Although the formation of polymeric Cr(III) under the condition of saturation have been investigated, these studies generally focused on their formation and physical or chemical properties in the pure solution with high Cr concentrations ranging from several µg L⁻¹ level to several thousands of µg L⁻¹ (Thompson and Connick, 1981; Spiccia and Marty, 1986; Rai et al., 1987; Stunzi et al., 1989; Saleh et al., 1996; Stewart and Olesik, 2000). Information on naturally occurring polymeric Cr(III) at µg L⁻¹ level was unknown due in part to the lack of reliable analytical method. In order to exam whether polymeric Cr(III) can occur more widely in the environment, samples from various sources, including CCA-treated wood leachates collected from the field experiment

for testing the leachability of As, Cr, and Cu associated with CCA-treated wood, waste water sample containing high concentration of Cr and a surface water from the Florida Everglades were analyzed for Cr speciation. Appreciable Cr content with various species was observed in CCA-treated wood leachates and waste water sample, but not in the wetland sample from Everglades. Concentrations and recoveries of representative samples are listed in Table 3. The wood leachates were analyzed within two days after collection from the field leaching system in order to preserve the integrity of Cr speciation in the field sample. However, the results for the waste water reflected the Cr speciation in the situation where samples were stored in the lab for a period of time (~ one month).

Polymeric Cr(III) was found to be the main Cr species in the wood leachate samples. The percentage of polymeric Cr (III) ranged from 39.1 to 67.4 % in these solutions, with a mass balance between 92.3 and 116.2%. Results of the wood leachate samples collected during a year-long field experiment (Hasan et al., 2010) indicate that the polymeric Cr(III) complexes always existed in wood leachates with total Cr concentration ranging from levels of low $\mu\text{g L}^{-1}$ to 1.7 mg L^{-1} . High percentage (84.3%) of polymeric Cr(III) was also observed in the waste water sample (Figure. 4). These results clearly suggest that polymeric Cr(III) complexes could exist in natural environment in certain circumstances. Our finding is also supported by a theoretical calculation of solubility of hydrous $\text{Cr}(\text{OH})_3(\text{s})$ in which polymeric Cr(III) (e.g. $\text{Cr}_3(\text{OH})_4^{5+}$) was found to be likely a dominant species in acidic solutions ($< \text{pH } 6.5$) (Stumm and Morgan, 1996). Therefore, speciation of Cr in the environmental samples by including only monomeric Cr(III) and Cr(VI) (Gomez and Callao, 2006) could underestimate the total Cr concentration in the samples. In addition, the existence of the polymeric Cr(III) at appreciable concentrations can alter the mobility, bioavailability and toxicity of Cr present in the environment.

CONCLUSIONS

We demonstrated that quantitative and simultaneous determination of several Cr species, including monomeric Cr(III), Cr(VI), and polymeric Cr(III) species in aqueous medium can be conducted using HPLC-DRC-ICPMS. Although the standard solution of polymeric Cr(III) is not easily prepared, it could be quantitatively estimated via the calibration curve of monomeric Cr(III). Results from different types of samples, including CCA-treated wood leachates and waste water revealed that polymeric Cr(III) could be one of the major forms of Cr existing at least in these type of natural environment even at $\mu\text{g L}^{-1}$ level of total Cr concentration. The existence of the polymeric Cr(III) in a broader natural condition would need further study to verify. Since the polymeric species could be the dominant form of Cr in some circumstances, caution should be paid to the selection of the method for Cr speciation in the environmental samples. Results obtained using methods that measure Cr(III) and Cr(VI) only could underestimate the total Cr concentrations and bias the Cr speciation results. The impact of polymeric Cr(III) on the environmental behavior and fate of Cr deserves further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- Aydin FA, Soylak M. Separation, preconcentration and inductively coupled plasma-mass spectrometric (ICP-MS) determination of thorium(IV), titanium(IV), iron(III), lead(II) and chromium(III) on 2-nitroso-1-naphthol impregnated MCl GEL CHP20P resin. *J Hazard Mater.* 2010; 173:669–674. [PubMed: 19765893]
- Bull DC. The chemistry of chromated copper arsenate - I. Characterisation of timber treatment plant chemical sludge. *Wood Sci Technol.* 2000; 34:367–376.
- Bull DC. The chemistry of chromated copper arsenate II. Preservative-wood interactions. *Wood Sci Technol.* 2001; 34:459–466.
- Bulut VN, Ozdes D, Bekircan O, Gundogdu A, Duan C, Soylak M. Carrier element-free coprecipitation (CEFC) method for the separation, preconcentration and speciation of chromium using an isatin derivative. *Anal Chim Acta.* 2009; 632:35–41. [PubMed: 19100880]
- Burbridge DJ, Koch I, Zhang J, Reimer KJ. Chromium speciation in river sediment pore water contaminated by tannery effluent. *Chemosphere.* 2012; 89:838–843. [PubMed: 22658944]
- Davis CM, Royer AC, Vincent JB. Synthetic multinuclear chromium assembly activates insulin receptor kinase activity: Functional model for low-molecular-weight chromium-binding substance. *Inorg Chem.* 1997; 36:5316–5320.
- Elci L, Divrikli U, Akdogan A, Hol A, Cetin A, Soylak M. Selective extraction of chromium(VI) using a leaching procedure with sodium carbonate from some plant leaves, soil and sediment samples. *J Hazard Mater.* 2010; 173:778–782. [PubMed: 19828238]
- Fukushima M, Nakayasu K, Tanaka S, Nakamura H. Chromium(III) binding abilities of humic acids. *Analytica Chimica Acta.* 1995; 317:195–206.
- Gomez V, Callao MP. Chromium determination and speciation since 2000. *Trac-Trends Anal Chem.* 2006; 25:1006–1015.
- Hasan AR, Hu LG, Solo-Gabriele H, Fieber L, Cai Y, Townsend TG. Field-scale leaching of arsenic, chromium and copper from weathered treated wood. *Environ Poll.* 2010; 158:1479–1486.
- Hingston JA, Collins CD, Murphy RJ, Lester JN. Leaching of chromated copper arsenate wood preservatives: a review. *Environ Poll.* 2001; 111:53–66.
- Hu LG, Diez-Rivas C, Hasan AR, Solo-Gabriele H, Fieber L, Cai Y. Transport and interaction of arsenic, chromium, and copper associated with CCA-treated wood in columns of sand and sand amended with peat. *Chemosphere.* 2010; 78:989–995. [PubMed: 20053417]
- IARC. IARC monographs on the evaluation of carcinogenic risks to humans. Chromium, Nickel and Welding. 1990; 49
- Jacquamet L, Sun YJ, Hatfield J, Gu WW, Cramer SP, Crowder MW, Lorigan GA, Vincent JB, Latour JM. Characterization of chromodulin by X-ray absorption and electron paramagnetic resonance spectroscopies and magnetic susceptibility measurements. *J Am Chem Soc.* 2003; 125:774–780. [PubMed: 12526678]
- Khan BI, Solo-Gabriele HM, Townsend TG, Cai Y. Release of arsenic to the environment from CCA-treated wood. 1 Leaching and speciation during service. *Environ Sci Technol.* 2006; 40:988–993. [PubMed: 16509347]
- Koshcheeva IY, Khushvaktova SD, Levinskii VV, Danilova VN, Kholin YV. Interaction of Cr(III) with the humus acids of soil, water, and bottom sediments. *Geochemistry International.* 2007; 45:178–184.
- Kotas J, Stasicka Z. Chromium occurrence in the environment and methods of its speciation. *Environ Pollut.* 2000; 107:263–283. [PubMed: 15092973]
- Krajnc M, Stupar J, Milicev S. Characterization of chromium and copper complexes with fulvic acids isolated from soils in Slovenia. *Science of the Total Environment.* 1995; 159:23–31.

- Laborda F, Gorris MP, Bolea E, Castillo JR. Mobilization and speciation of chromium in compost: A methodological approach. *Science of the Total Environment*. 2007; 373:383–390. [PubMed: 17215029]
- Levina A, Lay PA. Chemical properties and toxicity of chromium(III) nutritional supplements. *Chem Res Toxicol*. 2008; 21:563–571. [PubMed: 18237145]
- Ligang, Hu; Greer, Justin B.; Solo-Gabrele, Helena; Fieber, Lynne A.; Cai, Y. Arsenic toxicity in the human nerve cell line SK-N-SH in the presence of chromium and copper. *Chemosphere*. 2013; 91:1082–1087. [PubMed: 23473430]
- Metze, D.; Jakubowski, N.; Klockow, D. Speciation of chromium. In: Cornelis, R.; Crews, H.; Caruso, J.; Heumann, KG., editors. *Handbook of elemental speciation II: speciation in the environment, food, medicine & occupational health*. John Wiley & Sons, Ltd; 2005.
- Neubauer, K.; Reuter, W.; Perrone, P. Application Note. Perkin Elmer; 2003. Chromium speciation in water by HPLC/ICP-MS.
- Nico PS, Fendorf SE, Lowney YW, Holm SE, Ruby MV. Chemical structure of arsenic and chromium in CCA-treated wood: Implications of environmental weathering. *Environ Sci Technol*. 2004; 38:5253–5260. [PubMed: 15506225]
- Plaper A, Jenko-Brinovec S, Premzl A, Kos J, Raspor P. Genotoxicity of trivalent chromium in bacterial cells. Possible effects on DNA topology. *Chem Res Toxicol*. 2002; 15:943–949. [PubMed: 12119005]
- Qi WB, Reiter RJ, Tan DX, Garcia JJ, Manchester LC, Karbownik M, Calvo JR. Chromium(III)-induced 8-hydroxydeoxyguanosine in DNA and its reduction by antioxidants: Comparative effects of melatonin, ascorbate, and vitamin E. *Environ Health Perspect*. 2000; 108:399–402. [PubMed: 10811565]
- Rai D, Eary LE, Zachara JM. Environmental chemistry of chromium. *Sci Total Environ*. 1989; 86:15–23.
- Rai D, Sass BM, Moore DA. Chromium(III) hydrolysis constants and solubility of chromium(III) hydroxide. *Inorg Chem*. 1987; 26:345–349.
- Rao L, Zhang Z, Friesse JI, Ritherdon B, Clark SB, Hess NJ, Rai D. Oligomerization of chromium(III) and its impact on the oxidation of chromium(III) by hydrogen peroxide in alkaline solutions. *J Chem Soc, Dalton Trans*. 2002:267–274.
- Saleh FY, Mbamalu GE, Jaradat QH, Brungardt CE. Ion chromatography photodiode array UV-visible detection of Cr(III) hydrolytic polymerization products in pure and natural waters. *Anal Chem*. 1996; 68:740–745. [PubMed: 21619167]
- Santonen T, Zitting A, Riihimäki V, Howe PD, Wood M. Concise international chemical assessment document 76: Inorganic chromium(III) compounds. 2009
- Saputro S, Yoshimura K, Matsuoka S, Takehara K, Aizawa J, Tennichi Y. Speciation of dissolved chromium and the mechanism controlling its concentration in natural water. *Chem Geol*. 2014; 364:33–41.
- Seby F, Charles S, Gagean M, Garraud H, Donard OFX. Chromium speciation by hyphenation of high-performance liquid chromatography to inductively coupled plasma-mass spectrometry - study of the influence of interfering ion. *J Anal At Spectrom*. 2003; 18:1386–1390.
- Shrivastava HY, Ravikumar T, Shanmugasundaram N, Babu M, Nair BU. Cytotoxicity studies of chromium(III) complexes on human dermal fibroblasts. *Free Radical Biology and Medicine*. 2005; 38:58–69. [PubMed: 15589372]
- Song JK, Dubey B, Jang YC, Townsend T, Solo-Gabriele H. Implication of chromium speciation on disposal of discarded CCA-treated wood. *J Hazard Mater*. 2006; 128:280–288. [PubMed: 16165268]
- Spiccia L, Marty W. The fate of active chromium hydroxide, $\text{Cr}(\text{OH})_3 \cdot 3\text{H}_2\text{O}$, in aqueous suspension - study of the chemical changes involved in its aging. *Inorg Chem*. 1986; 25:266–271.
- Stewart II, Olesik JW. Investigate of Cr(III) hydrolytic polymerisation products by capillary electrophoresis-inductively coupled plasma-mass spectrometry. *Journal of Chromatography A*. 2000; 872:227–246. [PubMed: 10749500]

- Stewart MA, Jardine PM, Barnett MO, Mehlhorn TL, Hyder LK, McKay LD. Influence of soil geochemical and physical properties on the sorption and bioaccessibility of chromium(III). *J Environ Qual*. 2003; 32:129–137. [PubMed: 12549551]
- Stumm, W.; Morgan, JJ. *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters*. 3. John Wiley & Sons, Inc; 1996.
- Stunzi H, Marty W. Early stages of the hydrolysis of chromium(III) in aqueous solution. 1 Characterization of a tetrameric species. *Inorg Chem*. 1983; 22:2145–2150.
- Stunzi H, Spiccia L, Rotzinger FP, Marty W. Early stages of the hydrolysis of chromium(III) in aqueous solution. 4 Stability constants of the hydrolytic dimer, trimer, and tetramer at 25-degrees-C and I = 1.0 M. *Inorg Chem*. 1989; 28:66–71.
- Thompson M, Connick RE. Hydrolytic polymerization of chromium(III). 1 2 dimeric species. *Inorg Chem*. 1981; 20:2279–2285.
- US-EPA. SW 846, Method 7195, Chromium, hexavalent (coprecipitation). 1986a.
- US-EPA. SW 846: Method 7197, Chromium, hexavalent (chelation/extraction). 1986b.
- US-EPA. SW 846, Method 7196A, Chromium, hexavalent (colorimetric). 1992.
- US-EPA. SW 846, Method 7199, Determination of hexavalent chromium in drinking water, groundwater and industrial wastewater effluents by ion chromatography. 1996.
- Vincent JB. Elucidating a biological role for chromium at a molecular level. *Accounts Chem Res*. 2000; 33:503–510.
- Wolf RE, Morrison JM, Goldhaber MB. Simultaneous determination of Cr(III) and Cr(VI) using reversed-phased ion-pairing liquid chromatography with dynamic reaction cell inductively coupled plasma mass spectrometry. *J Anal At Spectrom*. 2007; 22:1051–1060.
- Yehiayan L, Pattabiraman M, Kavallieratos K, Wang X, Boise LH, Cai Y. Speciation, formation, stability and analytical challenges of human arsenic metabolites. *J Anal At Spectrom*. 2009; 24:1397–1405. [PubMed: 23495261]

Highlights

- 1.** Speciation of mono- and polymeric Cr(III), and Cr(VI) in aqueous samples.
- 2.** Polymeric Cr(III) could exist as a major form in aqueous samples containing $\mu\text{g L}^{-1}$ level of Cr.
- 3.** Methods that measure Cr(III)/Cr(VI) only could underestimate total Cr and bias Cr speciation.

Figure 1.

Mass spectrometric spectra of aged Cr(III) for 4 months obtained with electrospray ionization mass spectrometry. Both polymeric and monomeric Cr(III) could be observed. Signals of 1, 2, and 3 assigned to the monomeric Cr(III) species of $\text{Cr}(\text{OH})(\text{H}_2\text{O})_8^{2+}$ (107.8), $\text{Cr}(\text{OH})(\text{H}_2\text{O})_{10}^{2+}$ (125.6), and $\text{Cr}(\text{OH})_2(\text{H}_2\text{O})_5^+$ (177.7), respectively. Signals of 4 to 13 assigned to the polymeric Cr(III) of $[\text{Cr}_{2n+1}\text{O}_{2n-1}(\text{OH})_{2n+3}(\text{H}_2\text{O})_{n+6} - (n-1) \text{H}_2\text{O}]^{2+}$ ($n = 1, 2, 3, \dots$).

Figure 2.

Chromatograms of freshly prepared Cr(III), aged Cr(III), freshly prepared Cr(VI) and wood leachate obtained with HPLC-ICP-MS. These samples from different origins were diluted to a final concentration ranging from 50 to 200 $\mu\text{g L}^{-1}$ and were not at the same concentration. Post-column internal standard was used for each test.

Figure 3.

Chromatograms of wood extract (WE) spiked with Cr(III) (A), Cr(III) standard (B), WE spiked with Cr(VI) (C), and Cr(VI) standard (D). Note the scales of Y-axis for Cr(III) and Cr(VI) are different. The spiked concentration of Cr(III) and Cr(VI) in the original wood extract solutions were both $100 \mu\text{g mL}^{-1}$.

Figure 4. Chromatograms of the waste water collected from Ecuador. The spiked monomeric Cr(III) and Cr(VI) were both $50 \mu\text{g L}^{-1}$.

Table 1

Parameters used for HPLC and ELAN DRCE ICPMS systems

Parameters for HPLC system	
Column	PerkinElmer Spheri-5 RP-18 (220 × 4.6 mm)
Mobile phase	5% (v/v) methanol/0.5 mM EDTA + 1mM TBAH, pH = 7.2
Flow rate	1 mL min ⁻¹
Injection volume	100 µL

Parameters for ELAN DRCE ICP-MS system	
RF power	1350 W
Spray chamber	Quartz cyclonic spray chamber
Nebulizer	Meinhard nebulizer
Nebulizer gas flow	0.9 – 1.05 mL min ⁻¹
Lens voltage	7.0 – 9.0
Reaction gas	oxygen
Reaction gas flow	0.8 mL min ⁻¹
RPq	0.7
RPa	0
Total acquisition time	15 min

Table 2

Cr mass balance in different aged Cr(III) solutions with varied content of polymeric species

No	Percentage of polymeric Cr(III)	Mass balance
	%	%
1	12.2	96.4
2	8.4	106.6
3	12.7	102.8
4	12.7	103.8
5	9.7	111.2
6	14.9	108.6
7	13.9	89.6
8	12.8	94.8
9	17.9	100.9
10	11.3	103.7
11	12.4	98.1
12	16.7	95.3
13	19.0	94.3
14	11.7	80.7
15	21.5	91.8
16	10.0	87.0
17	8.9	96.9
18	5.6	89.9
19	6.2	94.1
Range	5.6 – 21.5%	80.7 – 111.0%

Table 3

Results of Cr speciation in typical samples with different origins

Sample	Species	Concentration ($\mu\text{g L}^{-1}$)	Recovery (%)	
			Cr(III)	Cr(VI)
CCA-treated wood leachate	Polymeric Cr(III)	14.1	93.3 ¹⁾	107.0 ¹⁾
	Monomeric Cr(III)	6.1		
	Cr(VI)	1.6		
Waste water	Polymeric Cr(III)	139.9	116.5 ²⁾	91.4 ²⁾
	Monomeric Cr(III)	16.2		
	Cr(VI)	9.7		
Deionized water	Polymeric Cr(III)	N.D.	86.2 ³⁾	106.8 ³⁾
	Monomeric Cr(III)	N.D.		
	Cr(VI)	N.D.		

¹⁾CCA-treated wood leachate spiked with $20 \mu\text{g L}^{-1}$ monomeric Cr(III) and $20 \mu\text{g L}^{-1}$ Cr(VI).

²⁾Waste water spiked with $50 \mu\text{g L}^{-1}$ monomeric Cr(III) and $50 \mu\text{g L}^{-1}$ Cr(VI).

³⁾Deionized water spiked with $50 \mu\text{g L}^{-1}$ monomeric Cr(III) and $50 \mu\text{g L}^{-1}$ Cr(VI)

N.D.: not detected (The calculated limit of detection for monomeric Cr(III) and Cr(VI) were all $0.9 \mu\text{g L}^{-1}$).