Simultaneous determination of chromium species by ion chromatography coupled with inductively coupled plasma mass spectrometry

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Abstract
Huge amounts of chromium (Cr) compounds are releasing into the environment because of anthropogenic activities. Speciation of chromium compounds is important in understanding the toxicity because of contrasting characters of hexavalent (Cr(VI) and trivalent Cr(III) chromium). An improved method for estimation of Cr species in environmental samples is developed using different complexing ligands and detected by ion chromatography (IC) coupled with inductively coupled plasma- mass spectrometer (ICP-MS). Among six complexing ligands probed, NTA $3Na^+$ and HEDTA gave the highest UV response and highest selectivity for Cr(III) complexation using an Agilent anion-exchange column with a mobile phase containing 20 mM NH_4NO_3 at pH 7.1 without Cl^- interference. Conditions including pH, concentration ratio [Cr(III) / NTA $3Na^+$, HEDTA)] and stability of Cr(III) complexes were investigated with pre-column derivatization. In conclusion, the proposed method could be used for the quantification of chromium species in contaminated water and soils within minutes.

Key Words
Chromium toxicity, speciation, ligands, complexation, retention time, IC-ICP MS.

Introduction
Chromium is used in many industries including electroplating, timber treatment, tannery industries, iron and steel industries (Nriagu and Pacyna 1988). As a result, anthropogenic discharge of chromium (Cr) often occurs into the environment. The toxicity, mobility and bioavailability of Cr depend on its oxidation state. Cr species exist in different oxidation states, from Cr^{2+} to Cr^{6+}. However, trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) are predominant species in environment. Hexavalent chromium (Cr(VI)) species present as chromate (CrO_4^{2-}), bichromate ($HCrO^{4-}$) and dichromate ($Cr_2O_7^{2-}$) which are toxic and mutagenic, mobile in alkaline and slightly acidic soils (Venitt and Levy 1974**;** Bauthio 1992). Nevertheless, $Cr^{3+}$is relatively non toxic and is considered as an essential nutrient at low levels in the human diet for effective glucose maintenance (Anderson 1989). Trivalent chromium [Cr(III)] mostly retained onto soil particles and also precipitated as Cr(III) hydroxide (Bolan *et al*. 2003).

Quantification of total chromium in environmental samples may not be sufficient to understand the distribution of various Cr species because of contrasting properties of Cr(III) and Cr(VI). Detailed information about oxidation states is essential to understand the toxicity of Cr. Therefore estimation of Cr(VI) and Cr(III) separately, rather than as total Cr is crucial. Several methodologies are available to measure Cr species in environmental samples using atomic absorption spectrometry (AAS) (Subramanian 1988; Sperling *et al*. 1992; James *et al.* 1995 and Adria-Cerezo *et al.* 2000). Ion chromatography (IC) coupled to inductively coupled plasma- mass spectrometry (ICP-MS) is currently available for metal speciation. However, the simultaneous determination of Cr(VI) and Cr(III) using a single chromatography column is difficult because Cr(III) is in the cationic form, however Cr(VI) is anionic . Hence, conversion of cationic Cr(III) to anionic form and subsequent separation of these two anionic species with a single anion-exchange chromatography hyphened with ICP-MS is possible since ICP-MS provides an ultra-sensitive elemental detector.

For separation of Cr(III) and Cr(VI) species, liquid chromatography (LC) coupled with inductively coupled plasma (ICP-MS) has been developed (Camara *et al*. 2000; Michalke 2002). Normally simultaneous determination of chromium is difficult because separation of Cr(VI) and Cr(III) using a single anionic column is impossible. However this problem can be resolved using two approaches. The first of these approaches uses an anion-exchange column and a cation-exchange guard column in series or a mixed mode column to retain both species (Pantsar-Kallio *et al.* 1996; Barnowski *et al.* 1997). The second approach

involves a derivatization process, whereby Cr(III) is complexed with a ligand to form anionic complex, which can be simultaneously separated from Cr(VI) by anion-exchange column on ion chromatography (Tomlinson *et al.* 1994; Byrdy *et al.* 1995). Aminopolycarboxylic acids like 1,2-cyclohexanediamine tetracetic acid (CDTA), ethylene diamine tetracetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA) were used to convert Cr(III) into anionic complexes (Jung *et al*. 1997; Himeno *et al.* 1998). The objectives of this work was to (i) identify the efficiency of complexation of different ligands with Cr(III); (ii) separation using ion chromatography (IC) and (iii) simultaneous determination of Cr(III) and Cr(VI) using ICP MS based on retention time.

Experimental

Agilent LC (1100 series) and ICPMS (Agilent 7500c) instruments were used for the determination of Cr(III) and Cr(VI). Agilent anion-exchange column (G3154A/102, 4.6x150mm) with a guard column (4.6x10mm) was used for chromatographic separation. The details of the instruments and the ICPMS conditions have been given elsewhere (Chen *et al.* 2007). Millipore water (18.2 MΩ/cm, Milli-Q plus system, Millipore, Bedford, MA, USA) was used throughout experimental procedure. Hexavalent chromium (hex chrome) standard solution (10 mM) was prepared from potassium dichromate (AR grade, Sigma-Aldrich, Sydney). Trivalent chromium stock standard solution (10mM) was prepared from chromium (III) nitrate, BDH, UK). Intermediate and working solutions were prepared daily.

Six ligands [PDA (2,6 pyridine dicarboxylic acid), HEDTA (N-(2-hydroxyethyl ethylene diamine triacetic acid), DTPA (diethylene triamine pentaacetic acid), EDTA (ethylene diamine tetraacetic acid, disodium salt), NTA (nitrilo triacetic acid), NTA 3Na+ (nitrilo triacetic acid, trisodium salt), AR grade, Sigma-Aldrich, Sydney] which varied in their complexing properties were taken in a beaker and mixed vigorously with Cr(III) at ratio of 1:2 with concentration of 0.1 mM. The mixed solution heated on hot plate at 80^0C for an hour to react Cr(III) with ligands because Cr(III) is kinetically inert at room temperature.
Solutions of Cr(VI) and [Cr(III)-ligand)] were mixed in a ratio of 1:1 in a glass vial and 20 µl of the solution was injected into HPLC (flow rate is 1.0ml/min at 30^0C column temperature) and NH_4NO_3 (10mM) was used as an eluent. Outlet of the separation column was connected directly to the nebuliser of ICP-MS via a polyetheretherketone (PEEK) tube and chromium was detected at *m/z* 52.

Results

Preliminary results indicated that except for NTA, all the five ligands chromatograms overlapped. To increase resolution further, solutions have been diluted to 0.01 mM different concentrations (10-30 mM) of mobile phase NH_4NO_3 were used and observed that retention time deceased with increasing concentration and both species separated best in 20 mM NH_4NO_3.

The results from ICP-MS chromatograms (Figure 1 and Figure 2) suggest that separation for Cr(III) and Cr(VI) was achieved using NTA $3Na^+$ and HEDTA as complexing agents. This complexation could be attributed to the formation of stable Cr-NTA $3Na^+$ and Cr-HEDTA, resulting in the separation based on difference in retention time, because the molar mass of [Cr(III) + ligand] is very much different from Cr(VI) species. Whereas the rest of the ligands have poor capabilities in complexing Cr(III). Temperature of the column, eluent pH and concentration ratio of Cr(III)/ NTA $3Na^+$, HEDTA were also investigated. Results indicated that maximum complexation of ligands with Cr(III) was achieved at pH 7.1 with a concentration ratio of 1:2 (Cr(III):ligands) at 80^oC for 20 min.

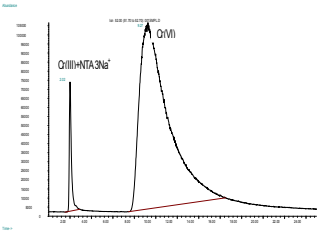

Figure 1. NTA $3Na^+$.

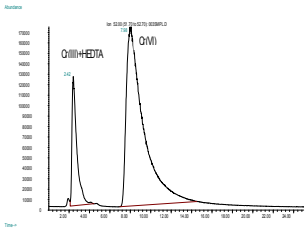

Figure 2. HEDTA.

Conclusions

The results suggest that stable chromium-chelate complexes, [Cr(III) – NTA] and [Cr(III) – HEDTA] were formed and successfully separated for both Cr(III) and Cr(VI) by complexing ligands based on difference in retention times on IC-ICP MS. This method could be used as a basis for separation and simultaneous quantification of Cr(III) and Cr(VI) in water and soil solutions. Further study is required to determine the stability constants of Cr(III) – NTA] and [Cr(III) – HEDTA] complexes.

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