Methodology for the Speciation of Cr(III) and Cr(VI) in Leaf Powders Using the Sulfate Form of Dowex-1 After Microwave Extraction and ICP-OES Detection

P. Mamatha^a, G. Venkateswarlu^b, S. Thangavel^b, A. C. Sahayam^{*,b,c}, and A.V.N. Swamy^b

^a JNTUACEP, Pulivendula, Kadapa-516390, India

^b National Centre for Compositional Characterisation of Materials (NCCCM)
Bhabha Atomic Research Centre (BARC), ECIL (P.O.), Hyderabad-500062, India

^c Homi Bhabha National Institute (HBNI), Mumbai, India

INTRODUCTION

Chromium (Cr) is a controversial element and is both essential and toxic, depending on its different species. Chromium is the most prevalent and reactive pollutant which dissipates into the ecosystem from a variety of industrial activities such as electroplating, leather tanning, mining, textile dyeing, wood preserving, chromate preparation, and metal finishing (1). As a result, chromium has become one of the major contaminants to the environment. It is considered essential or toxic depending on the daily intake, oxidation state, and bio-availability (2, 3). Tea (black and green) is an aromatic beverage widely consumed worldwide and is made from dried leaves of *Camellia sinensis*. Drinking tea might help in reducing serum cholesterol, provide anti-aging conditions, and decrease the risk of cardiovascular disease and cancer, has less caffeine than coffee, is good for the heart and bones (4). In tea there are compounds such as fluoride, and essential minerals that are beneficial to human health (5). However, the presence of toxic trace elements in tea and vegetables are due to acidic or contaminated soils containing bio-available metals for root uptake (6, 7). As a result, the trace element content in tea may have beneficial or adverse effects on human health. For example in the case of Cr, Cr(III) is essential, while Cr(VI) is toxic.

ABSTRACT

The speciation of chromium (Cr) in different leaf samples from black tea, green tea, spinach, the *Ceiba pentandra* and *Croton escabiosis* fruit trees, and one custard apple tree (*Annona reticulata*) is reported by using off-line separation and ICP-OES detection. The species of Cr are extracted using a solution containing 0.1 M EDTA, 1% tetrabutyl ammonium bromide (TBAB), and a little HF in a domestic microwave oven for 1 minute. Optimization of the methodology for the extraction of the Cr species from dried leaf powders and separation of Cr(VI) from the extract were studied. The determination of the separated Cr(VI) and total Cr in the extracts and also total Cr after total digestion was carried out using ICP-OES. Recovery of the extracted Cr was 97-103%, calculated with respect to the total Cr concentration obtained after complete dissolution. Separation of Cr(VI) from the extracts was carried out using anion exchange chromatography with a sulfate form of Dowex-1 at pH 4.5. The recovery of Cr(VI) was found at 98-101% with an RSD of 5-6 %. The limits of detection for Cr(III) and Cr(VI) were 18 and 21 ng g⁻¹, respectively. The analytical methodology thus developed was validated for total chromium using a Polish certified reference material Virginia Tobacco Leaves (CRM-CTA-VTL-2). The sum of the concentrations of Cr(VI) and Cr(III) was in good agreement with the total Cr in the CRM and with the total Cr determined after total digestion.

Hence, it is necessary to know the concentration of Cr(VI) in tea. Similarly, Cr is also present in green leafy vegetables, such as spinach (*Palak*). Even in this case, the presence of Cr(VI) poses adverse health effects (8, 9). The toxic nature of Cr(VI) is attributed to its higher oxidation potential and relatively smaller size, which enables it to penetrate biological cell membranes. Consumption of excess Cr(VI) has a bad impact on the liver, lungs, and kidneys and causes cancer by oxidizing biological species, such as the DNA and some proteins. The speciation of Cr is also important in leaves that have medicinal or nutritional uses. Thus, to evaluate the biological effects of Cr on humans or its potential toxicity, speciation analysis is required. The importance and the need for adequate analytical tools for Cr speciation was also emphasized in a review article by Scancar and Milacic (10).

For speciation analysis, the Cr species need to be extracted from the solid samples prior to their determination without any potential for interconversion. Alkali leaching has been reported for the speciation of Cr in foodstuff (11-13). Microwave-assisted digestion has gained wide acceptance as a rapid method for sample decomposition in inorganic analysis. Recently, it has also been verified as an appropriate tool for the rapid preparation of solid samples for organometallic speciation analysis (14). Jiang et al. reported microwave-assisted extraction of Cr(III) and Cr(VI) from soil and sediments using TBAP, EDTA, and HF (15). A similar procedure was reported

* Corresponding author.
E-mail: sabayamac@gmail.com
Tel: 91-40-27121365
Fax: 91-40-27125463

by the authors for the off-line speciation of Cr (16).

The aim of the present work was to develop a rapid microwave-assisted extraction procedure for Cr(III) and Cr(VI) suitable to a wide variety of leaves, and also offline ion exchange separation and determination of Cr(III) and Cr(VI) in different types of leaf powders using a sulfate form of Dowex-1. Optimization of the conditions for quantitative separation of Cr(III) and Cr(VI) and the determination of these species by inductively coupled plasma optical emission spectrometry (ICP-OES) is reported.

EXPERIMENTAL

Instrumentation

A Model ULTIMA 2 ICP-OES (Horiba JobinYvon, Longjumeau Cedex, France) was used for this study. For the extraction of Cr(III) and Cr(VI) from selected leaf powders, a domestic microwave oven (Videocon, India) was used. The operating conditions of the ICP-OES instrument and the microwave oven are given in Table I.

TABLE I
Operating Conditions of ICP-OES and Microwave Oven

Parameters	
ICP-OES	
Power	1.1 kW
Wavelength	283.563 nm
Plasma gas flow rate	12.1 L min ⁻¹
Nebulizer type	Concentric
Spray chamber	Cyclonic
Nebulizer gas flow rate	0.83 L min ⁻¹
Auxiliary gas flow rate	0.52 L min ⁻¹
Microwave Oven	
Power (700 watt)	1 min

Standard Solutions, Reagents and Chemicals

Throughout the study, analytical grade reagents were used. Pure water (18M Ω .cm) used for dilutions was prepared from a Milli-Q[®] water purification system (Millipore Corporation, USA) with feed water obtained from a mixed-bed ion exchanger. A Dowex-1 resin (chloride form) was obtained from Sigma, St. Louis, MO, USA. The Cr(III) and Cr(VI) standards were prepared using AR grade Cr(III) chloride and potassium dichromate, respectively. HF and EDTA were procured from Merck (Darmstadt, Germany) and tetra butyl ammonium bromide from Sisco Research Laboratories, India. The polypropylene bottles used for sample collection were soaked overnight in 20% nitric acid, cleaned thoroughly with deionized water to remove the acid, then rinsed with Milli-Q water. EDTA (0.1 M) and TBAB (1%) were prepared in Milli-Q water.

Preparation of Resin

Dowex-1 resin (10 g chloride form, 100–200 mesh) was placed into a 50-mm i.d. column, equilibrated with 2M HNO₃, washed to remove the acid with Milli-Q water, and equilibrated with HCl. The sulfate form of Dowex-1 was prepared by passing 100 mL of 10% potassium sulfate through this resin to convert it into sulfate form, washed, air-dried, and used for subsequent studies. The flow rate used for the above experiments was 1 mL min⁻¹.

Adsorption

A glass column of 10 mm i.d. was prepared by adding 2 g of the sulfate form of Dowex-1. Leaf powder extracts, containing Cr(III) and Cr(VI), were passed through the resin at a flow rate of 1 mL min⁻¹ after adjusting the pH ranging from 2.5–6.5. The eluate was analyzed for Cr(VI). The pH of the loading solutions was adjusted using dilute HCl and NH₄OH solutions.

Elution

After adsorption, the adsorbed Cr(VI) was eluted using 20 mL of different concentrations of HNO₃ (0.5–2.5 M) at a flow rate of 1 mL min⁻¹. The eluate was analyzed for Cr(VI).

Samples

Tea powders, two black and one green tea, and three different spinach (palak) samples were purchased from local markets and used as procured. Other leaf samples such as *Ceiba pentandra*, *Croton escabiosis*, and custard apple tree leaves were collected near the site of the Uranium Corporation of India (UCIL), located in Tummala-palli, Kadapa, India.

Sample Preparation, Extraction, and Analytical Procedure

All leaf samples were washed to remove the dust, then air-dried, and finally dried at 70 °C. The dried leaves were ground and sieved to the particle size of 75 μ m., then used for extraction of Cr(III) and Cr(VI) by the microwave-assisted extraction procedure. The leaf powder samples (0.1 g) were put into polyethylene tubes and 3 mL 0.1 M EDTA, 3 mL of 1% tetrabutyl ammonium bromide, and 250 μ L of hydrofluoric acid (48%) added, then placed into a fume hood wearing hand gloves to protect the analyst from corrosive chemicals (15, 16). The tube was kept in a glass beaker containing water and exposed to the microwaves. The temperature program used was set at the power of 700 W and a time exposure of 1 minute (Table I). After cooling, the mixture was centrifuged at 1500 rpm, the supernatant taken into 10-mL volumetric flasks, and diluted to the mark with rinsing water (2 mL). The extract was then used for ion exchange separation of Cr(III) and Cr(VI).

The extractant solution was transferred to a beaker and the

pH adjusted to 4.5, then passed through the column packed with 2 g of the sulfate form of Dowex-1 resin at a flow rate of 1 mL min⁻¹. Cr(VI) was adsorbed onto the resin, eluting out Cr(III). The adsorbed Cr(VI) was eluted with 2M HNO₃. Both solutions were then analyzed for Cr(III) and Cr(VI) by ICP-OES.

To verify the recoveries of the species by microwave extraction and also to check the sum of Cr(III) and Cr(VI) with total Cr, the leaf powders were completely digested by closed microwave digestion using the required HNO₃. All sample solutions were analyzed for total Cr.

RESULTS AND DISCUSSION

A direct determination of Cr in the leaf sample solutions by ETAAS, ICP-OES, or ICP-MS will not offer any information about the chemical species of Cr. Hence, the separation of species either on-line or off-line prior to the determination is necessary. However, the species of interest needs to be brought into solution from the solid samples without altering the species. During complete dissolution, the sample undergoes harsh conditions that may lead to interconversion or decomposition of the species. Leaching of species using mild reagents and conditions was found

to be suitable and is being investigated further (17, 18). For the speciation of Cr from solid samples, sample preparation was achieved by alkali leaching or microwave leaching in combination with EDTA and TBAC/ TBAB. In the present study, leaching of Cr(III) and Cr(VI) was attempted by using EDTA and TBAB. HF was found to be necessary for the quantitative extraction of both species (15). In the extracts, hexavalent chromium exists in the form of ion pair (HCrO₄)⁻(TBAB)⁺ and hence, can be selectively adsorbed on anion exchange resins for the separation of Cr(III) and Cr(VI). The Dowex-1 resin is reported to be the best suited resin for the anion exchange separation of Cr(VI) (19). Though the resin is found to have preferential adsorption for Cr(VI) quantitatively, our earlier experience with the chloride form of Dowex-1 inferred the leaching of the adsorbed Cr due to the reduction of Cr(VI) to Cr(III) (20). Hence, the sulfate form of the Dowex-1 resin was chosen since it alleviates the problem and also reduces interferences from other singly charged anions present in real sample extracts because the counter ion is doubly charged. The pH of the loading solution and also the concentration of the eluting reagent were optimized to achieve maximum recovery.

Optimization Studies

To obtain quantitative recoveries during separation of Cr(III) and Cr(VI), the pH of the loading solution and the concentration of HNO₃ to elute the adsorbed Cr(VI) need to be optimized. The studies were carried out using standard solutions of Cr(III) and Cr(VI) at 250 ng mL⁻¹ concentration.

Adsorption

The effect of the pH of the sample solution on the adsorption of Cr(III) and Cr(VI) was studied by changing the pH from 2.5–6.5 for both Cr(III) and Cr(VI). As shown in Figure 1, for the selective adsorption of Cr(VI) and the rejection of Cr(III) a pH of 4.5 was found to be suitable for this study. Elution of the adsorbed Cr(VI) was carried out using 2M HNO₃. Though there is little sorption of Cr(III), this pH was selected because of the large separation factor, the possibility of reducing Cr(VI) to Cr(III) at a lower pH in the presence of organic materials, and the ability to obtain better recovery.

Elution

Elution was carried out with HNO₃ and the concentration required was in the range 0.5 to 2.5 M for a 20-mL eluting solution. The results indicated that near quantitative recoveries were obtained when 2 M HNO₃ was used for the elution (Figure 2).

The anion exchange method adopted could separate Cr(VI) in the presence of Cr(III). However, there is a risk of competition from other anions present in the sample extract. There is no significant interference from anions such as Cl⁻, NO₃⁻, and HCO₃⁻ possibly due to the preference of doubly charged ions in comparison to singly charged ions.

Fig. 1. Recovery of Cr(VI) with pH of loading solution.

Limits of Detection

The limits of detection, calculated as three times the standard deviation of blank measurements ($n=6$), were 18 and 21 ng g⁻¹, respectively, for Cr(III) and Cr(VI). The limits of detection obtained were found to be superior or close to reported procedures based on a similar strategy (21–25).

Validation of Methodology

Since the recoveries of both Cr(III) and Cr(VI) are near quantitative, the procedure was applied using the CRM-CTA-VTL-2 Virginia Tobacco Leaves to ensure accuracy. The sum of concentrations of Cr(III) and Cr(VI) was in close agreement with the certified total concentration of Cr (Table II). The procedure was also applied to two tea and one green tea powders. Even in case of the tea samples, the sum of the concentration of both Cr species was in close agreement with the total Cr determined by ICP-OES after complete dissolution of the samples (see Table II).

Speciation of Cr(III) and Cr(VI) From Leaf Samples

The validated analytical methodology developed was also applied to leaf samples from three types of spinach, the *Ceiba pentandra* and *Croton escabiosis* fruit trees, and

one custard apple tree (see Table III). The concentration of Cr(VI) in all samples analyzed in this study was below the detection limits of ICP-OES indicating that the total Cr and Cr(III) concentrations are almost the same. This observation is in accordance with Novotnik et. al. (11) and Mandivana et. al. (26).

CONCLUSION

The sulfate form of Dowex-1 resin was found to be suitable for the speciation of Cr(III) and Cr(VI) in different leaf samples. The recovery of Cr(VI) was found to be 98–101% with an RSD of 5–6%. The developed methodology was applied for the speciation of Cr in tea products (black and green), spinach, and fruit tree leaves. The method is simple, straightforward, the consumption of reagents is low, the resin can be reused and enables

the independent determination of Cr(III) and Cr(VI), as well as being suitable for the routine monitoring and validation of Cr(VI) in the CRM Virginia Tobacco Leaves.

ACKNOWLEDGMENT

The authors thank Dr. Sunil Jai Kumar, Head, NCCCM, for his support and encouragement in carrying out this work.

Received June 22, 2018.

REFERENCES

1. L. Hu, Y. Cai and G. Jiang, *Chemosphere* 156, 14 (2016).
2. A.N. Modenes, A. Paula de Oliveira, F. R. Espinoza-Quinines, D.E.G. Trigueros, A. D. Kroumov and R. Bergamasco, *Chemosphere* 172, 373 (2017).

TABLE II
Concentration of Cr(III) and Cr(VI) in CRM and Tea Powders^a

Sample	Cr(III) (mg/kg)	Cr(VI) (mg/kg)	Total Cr (mg/kg)
(CRM-CTA-VTL-2)			
Virginia Tobacco Leaves	2.0 ± 0.2	<0.05	1.87 ± 0.16 ^b
Tea powder 1	2.3 ± 0.2	< 0.05	2.2 ± 0.2
Tea powder 2	1.2 ± 0.1	< 0.05	1.1 ± 0.1
Green Tea	0.6 ± 0.1	< 0.05	0.6 ± 0.1

^a Results are mean of three experiments ± standard deviation.

^b Certified.

Fig. 2. Elution of Cr(VI) with concentration of HNO₃.

TABLE III
Concentration of Cr(III) and Cr(VI) in Different Leaf Samples^a

Sample	Cr(III) (mg/kg)	Cr(VI) (mg/kg)
Spinach 1	2.5 ± 0.2	<0.05
Spinach 2	3.7 ± 0.3	<0.05
Spinach 3	5.5 ± 0.3	<0.05
<i>Ceiba Patandra</i>	0.2 ± 0.1	<0.05
<i>Croton Scabiosis</i>	0.7 ± 0.1	<0.05
Custard Apple Tree	1.5 ± 0.1	<0.05

^a Results are mean of three experiments ± standard deviation.

3. K. Pyrzynska, Trends Anal. Chem. 32, 100 (2012)
4. F. L. Chung, J. Schwartz, C. R. Herzog and Y. M. Yang, J. Nutr. 133, 3268S (2003).
5. C. Cabrera, R. Gimenez and M. C. Lopez, J. Agric. Food Chem. 51, 4427 (2015).
6. W. Han, F. Zhao, Y. Shi, L. Ma and J. Ruan, Environ. Pollut. 139, 125 (2006).
7. G. Nabulo, C. R. Black and S. D. Young, Environ. Pollut. 159, 368 (2011).
8. <http://www.webmd.com/digestive-disorders/tc/chromium-topic-overview>
9. Y.-P. Liao, Z.-X. Wang, Z.-H. Yang, L.-Y. Chai, J.-Q. Chen and P.-F. Yuan, Trans. Nonferrous Met. Soc. China 21, 2520 (2011).
10. J. Scancar and R. Milacic, J. Anal. At. Spectrom. 29, 427 (2014).
11. B. Novotnik, T. Zuliani, J. Scancar and R. Milacic, J. Anal. At. Spectrom. 28, 558 (2013).
12. K. I. Mandiwana, N. Panichev and S. Panicheva, Food Chem. 129, 1839 (2011).
13. M. E. Soares, E. Vieira and M. D. L. Bastos, J. Agric. Food Chem. 58, 1366 (2010).
14. C.M. Tseng, A. De Diego, F.M. Martin and O.F.X. Donard, J. Anal. At. Spectrom. 12, 629 (1997).
15. C.-Y. Kuo, S.-J. Jiang and A. C. Sahayam, J. Anal. At. Spectrom. 22, 636 (2007).
16. P. Mamatha, G. Venkateswarlu, A. V. N. Swamy and A. C. Sahayam Anal. Methods 6, 9653 (2014).
17. U. Araujo-Barbosa, E. Peña-Vazquez, M. C. Barciela-Alonso, S. L. C. Ferreira and P. Bermejo-Barrera, Talanta 170, 523 (2017).
18. L.-F. Chang, S.-J. Jiang and A. C. Sahayam, J. Chromatogr. A 1176, 143 (2007).
19. S. Edebalı and E. Pehlivan, Chem. Eng. J. 161, 161 (2010).
20. A.C. Sahayam, G. Venkateswarlu and S.C. Chaurasia, Anal. Chim. Acta 537, 267 (2005).
21. S. Sahan, S. Sacmaci, S. Kartal, M. Sacmaci, and U. Sahin, Talanta 120, 391 (2014).
22. K. O. Segi, M. Tuzen, M. Soylak, and L. Elci, J. Hazard. Mater. 153, 1009 (2008).
23. K. Kiran, K. S. Kumar, B. Prasad, K. Suvardhan, L. R. Babu, and K. Janardhanam, J. Hazard. Mater. 150, 582 (2008).
24. F. A. Aydin and M. Soylak, J. Hazard. Mater. 162, 1228 (2009).
25. M. S. Hosseini and ARR Sarab, Int. J. Environ. Anal. Chem. 87, 375 (2007).
26. K.L. Mandiwana, N. Panichev, M. Kataeva, and S. Siebert, J. Hazard. Mater. 147, 540 (2007).

❖ CRC Press/Taylor & Francis announces the availability of ❖

Measuring Elemental Impurities in Pharmaceuticals: A Practical Guide

**Robert J. Thomas, CSci, CChem, FRSC Principal Consultant
Scientific Solutions Gaithersburg, MD**

- The complete guide to measuring elemental impurities in pharmaceutical and nutraceutical materials.
- Covers heavy metals testing in the pharmaceutical industry from an historical perspective.
- Gives an overview of current USP Chapters <232> <233> and <2232> and ICH Q3D Step 4 Guidelines.
- Explains the purpose of validation protocols used in Chapter <233>, including how J-values are calculated
- Describes fundamental principles and practical capabilities of ICP-MS and ICP-OES.
- Offers guidelines about the optimum strategy for risk assessment.
- Provides tips on how best to prepare and present your data for regulatory inspection.

Online www.crcpress.com