

Speciation of Cr(III) and Cr(VI) in surface waters with a Chelex-100 resin column and their quantitative determination using inductively coupled plasma mass spectrometry and instrumental neutron activation analysis

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Inorganic Cr(III) and Cr(VI) have contrasting biological, geochemical and toxicology effects. Cr(III) is considered as an essential species for the proper functioning of living organisms but Cr(VI) is toxic for the biological systems. An off-line speciation method using Chelex-100 has been practiced for speciation to Cr(III) and Cr(VI) from surface waters of rivers. The underlying principal of this separation method is based on the ability of cationic Cr(III) to be retained by the resin Chelex-100 while the anionic Cr(VI) remained in the sample matrices. The efficiency of this technique was improved by studying the effect of resin pH. Quantitative determination using inductively coupled plasma-mass spectrometry (ICP-MS) and instrumental neutron activation analysis (INAA) was carried out after the separation to determine the total Cr and Cr(VI) in the liquid matrices. The precision and the accuracy of the quantitative analysis were evaluated by using standard reference material NRCC CASS-2. Intercomparison of INAA and ICP-MS results were determined. The quantity of inorganic Cr(III) and Cr(VI) in the surface water of rivers in the vicinity of industrial areas was investigated together with the determination of the physical properties of the water rivers during sampling.

Introduction

The interest in inorganic chromium speciation originates from the widespread use of this metal in electroplating, tanning and dyeing industry inducing more and more Cr compounds to be discharged into the natural water systems which could ultimately have significant adverse biological and ecological effects. Therefore, the quantity of inorganic Cr has been identified as an important water quality index.

The two common oxidation states of Cr present in natural water are Cr(III) and Cr(VI). Both Cr(III) and Cr(VI) have contrasting biological, geochemical and toxicology effects.¹ Cr(III) is considered to be a trace element species essential for the proper functioning of mammals.² It is able to maintain the metabolism of glucose, lipids and proteins, whereas Cr(VI) can induce toxic effects on biological systems and has the ability to permeate biological membranes. Inhalation and retention of Cr(VI)-containing materials can cause the increased incidence of bronchogenic carcinoma. Skin contact of Cr(VI) compounds can lead to skin allergies and cancer.³

Total Cr measurement may not be suitable to evaluate actual environmental impact due to the considerable difference in toxicology of these two elemental forms. Therefore, speciation of Cr(III) and Cr(VI) are important. Speciation analysis can be performed either using on-line or off-line techniques. The traditional off-line method involved the separation or preconcentration of a particular species before manual transport of the processed samples to the instrument for quantitative measurement. Traditionally, Cr species

were separated and enriched by chelating resin,⁴ colored complex formation,⁵ electrochemical method,⁶ coprecipitation,⁷ ion-exchange resin.⁸ The chelating resin, Chelex-100 has frequently been applied for the separation and preconcentration of trace metal ions because it readily forms stable chelates.⁹ An application of Chelex-100 for the separation of Cr(III) and Cr(VI) has been reported in which the separation technique involved an automated cationic column and anionic column. Chelex-100 was used as a cationic-exchanger to retain Cr(III) while Cr(VI) was retained on an anionic column.¹⁰ This concept was applied in this study in the speciation of Cr(III) and Cr(VI) from surface waters.

The Cr concentration in rivers is usually limited to 0.5–100 nM.¹¹ A sensitive and accurate technique is required for the determination of the separated Cr species due to its low concentration occurrence in the processed samples. Recently, inductively coupled plasma mass spectrometry (ICP-MS) has played an important role in the modern analytical laboratory because of its excellent sensitivity, precision and accuracy.^{12,13} It has been identified as a suitable detector for speciation analysis due to its low detection limit. However, ICP-MS still suffers in comparison to instrumental neutron activation analysis (INAA) with respect to its potential for sample contamination during the required sample dissolution, its inherently destructive nature, problems with precision (especially in the presence of certain high-concentration elements and with variable blanks are encountered), and spectral interferences in the argon plasma due to the presence of high chloride and sulfate levels.¹⁴ Moreover, INAA is a comparable analysis technique to ICP-MS due to its

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ability to determine multi-elements at the same time and its low detection limit. Besides, chromium can be accurately determined by INAA (high cross section and well identified gamma-rays).¹⁵ Hence, INAA was performed as the comparable technique for ICP-MS quantitative analysis. These combinations of techniques offer significant advantages such as reproducibility and minimal interference and low detection limit (ultra trace level) for ICP-MS although it is time consuming and needs high attention.

Experimental

Sampling storage and column preparation

The study was confined to the industrial area of Johor Bahru and Pasir Gudang in the state of Johore, Malaysia. There are five rivers chosen within the study area with three sampling sites designated for each river. The water samples were collected at a depth of about 30 cm from the surface water of the rivers and kept in polyethylene (PE) bottles and later filtered through 0.45 μm Millipore membrane filters supplied by Whatman. Preservation of water samples using HNO_3 is not recommended because the addition of acid solution will change the oxidation state of Cr species in the samples.¹⁶

All reagents used were of analytical grade. Deionized water from Nanopure system was used for all aqueous solution preparations. Stock solutions of 1000 $\text{mg}\cdot\text{l}^{-1}$ Cr(III) and Cr(VI) were prepared by the dissolution of $\text{Cr}(\text{NO}_3)_3\cdot 9\text{H}_2\text{O}$ (Merck) and $\text{K}_2\text{Cr}_2\text{O}_7$ (Merck), respectively. Standard Cr(III) and Cr(VI) solutions of lower concentrations were prepared by the dilution of the stock solution. Chelex-100 resin (iminodiacetate group) of 50–100 mesh size in the Na^+ form was purchased from Sigma. All containers were washed by soaking them with Triton X100 followed by 10% HNO_3 solution for at least 24 hours. The apparatus was then rinsed with deionized water prior to drying in the oven.

Column speciation

For a 100 ml river water sample, 1 g of resin was weighed and mixed with small amount of water and the slurry was poured into the column. The column consisted of a 15 cm height and 0.5 cm i.d. Pyrex glass with a reservoir of approximately 120 ml capacity from the top of the column. The slurry of the resin was washed with 15 ml of 2.5M HNO_3 to remove metal impurities present in the resin. Then, 10 ml of deionized water was used to rinse the excess acid. To transform the resin to the NH_4^+ form, 10 ml of 2.0M NH_4OH was added. After checking the pH of the effluent to ensure

the basicity, the column was then rinsed with 10 ml of deionized water to remove excess NH_4OH . The pH of 100 ml river water sample was adjusted to 4 with dropwise addition of NH_4OH or HNO_3 . Then, 0.5 ml of 8M ammonium acetate solution (Merck) was added to aid the buffering in the system. A small amount of the sample was first added to the column to allow the resin to undergo its natural shrinkage. After complete transformation was observed, the remaining sample was added to the reservoir and the flow rate of the samples was adjusted to approximately $0.5\text{ ml}\cdot\text{min}^{-1}$. The eluant was then kept in PE bottle for further quantitative analysis. Cr(III) and Cr(VI) standard solutions were used to optimized the column speciation.

Inductively coupled plasma mass spectrometry (ICP-MS)

The ICP-MS instrument used in all experiments was the Elan 6100 model purchased from Perkin Elmer Corporation. The operating parameters are described in Table 1. A quantitative analysis mode (QTM) was used for the data acquisition of the samples. The scanning of each sample was repeated three times to gather reasonably good statistics. A pulse counting was used during the analysis. The filtered river waters and the column eluants were analyzed using ICP-MS with the optimum conditions to determine the total Cr and Cr(VI) concentration, respectively.

Instrumental neutron activation analysis (INAA)

Instrumental neutron activation analysis was carried out at the Malaysian Institute of Nuclear Technology (MINT). About 0.15 g of the processed resin was weighed into a 55 mm \times 15 mm (id) PE vial. The weight was recorded and the vial was heat-sealed. The sealed vial was placed in a PE capsule and sealed together with three sub-samples prepared for each sample. For liquid samples, about 0.3 ml of the liquid samples were spiked into a weighed wool which was placed into the PE vials. Irradiation was carried out in a thermal neutron flux of $4.0\cdot 10^{12}\text{ n}^{-1}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ from a TRIGA Mark II reactor running at 750 kW for 6 hours. The standard reference material CASS-2 was irradiated together with the samples to eliminate the systematic error during irradiation.

Table 1. Operating parameters of ICP-MS

Parameter	Current value
Nebulizer gas flow rate	0.84 l/min
Lens voltage	6.50 V
ICP RF power	1014.74 W
Analog stage voltage	-2033.57 V
Pulse stage voltage	1226.22 V
Monitoring mass	<i>m/z</i> 52

The cooling time for ^{51}Cr was 27.8 days. After the cooling period, the concentrations of the samples were determined by investigating the γ -rays of ^{51}Cr species via the 320.23 keV photopeak of ^{51}Cr using a coaxial HPGe detector. The counting of each sample was performed based on 3600-seconds counting time. The detector had a resolution of 1.9 keV at 1332 keV and a relative efficiency of 20%. The detector was connected to an Ortec 472A spectroscopy amplifier and a Nuclear Data ND 66 multi-channel analyzer calibrated at 0.5 keV per channel. A dedicated PDP11 computer connected to the system was used in the identification and quantization steps.

Certified standard reference material (CRM)

A high degree of analytical accuracy is required for environmental analysis. The accuracy of the results strongly depends on the quality of the analysis of the reference material. Therefore, in this study, a certified standard reference material (CASS-2) has been utilized to establish the quality control. The certified value of total Cr in the SRM CASS-2 is $0.092 \pm 0.006 \mu\text{g}\cdot\text{g}^{-1}$.

Results and discussion

The 15 river sites chosen are shown in Fig. 1. All the 15 sites chosen are situated in industrial areas to

investigate the pollution status of the rivers due to the presence of excess Cr. The river quality parameters are shown in Table 2. The principle of the Cr speciation technique using Chelex-100 is based on the high affinity of cationic Cr(III) retention on Chelex-100 while the anionic Cr(VI) remained in the liquid matrices which was further analyzed by ICP-MS.¹⁷ While the Cr(III) in the resin was determined using INAA.

The efficiency of Chelex-100 depends on the pH value of the resin itself because the pH of the resin will affect the binding site of the resin for metal adsorption of interest. A study was carried out to evaluate the effect of pH and on the separation of Cr(III) and Cr(VI) and to ensure the optimum conditions for this separation method. The retention of Cr(III) on Chelex-100 was found to be high and the adsorption of Cr(VI) was the least when the pH condition of the resin was changed to pH 4 (Fig. 2). Below pH 3.0, Chelex-100 is known to be an anionic exchanger and the chelation was inhibited by the high concentration of hydrogen ions. Above a pH of 8.0, there was incomplete collection of Cr(III) due to the formation of chromium hydroxide species such as $\text{Cr}(\text{OH})_2^{2+}$ and $[\text{Cr}(\text{OH})_2]^+$.¹⁸ Therefore, the pH 4.0 acetate buffers were selected for the sample analysis.

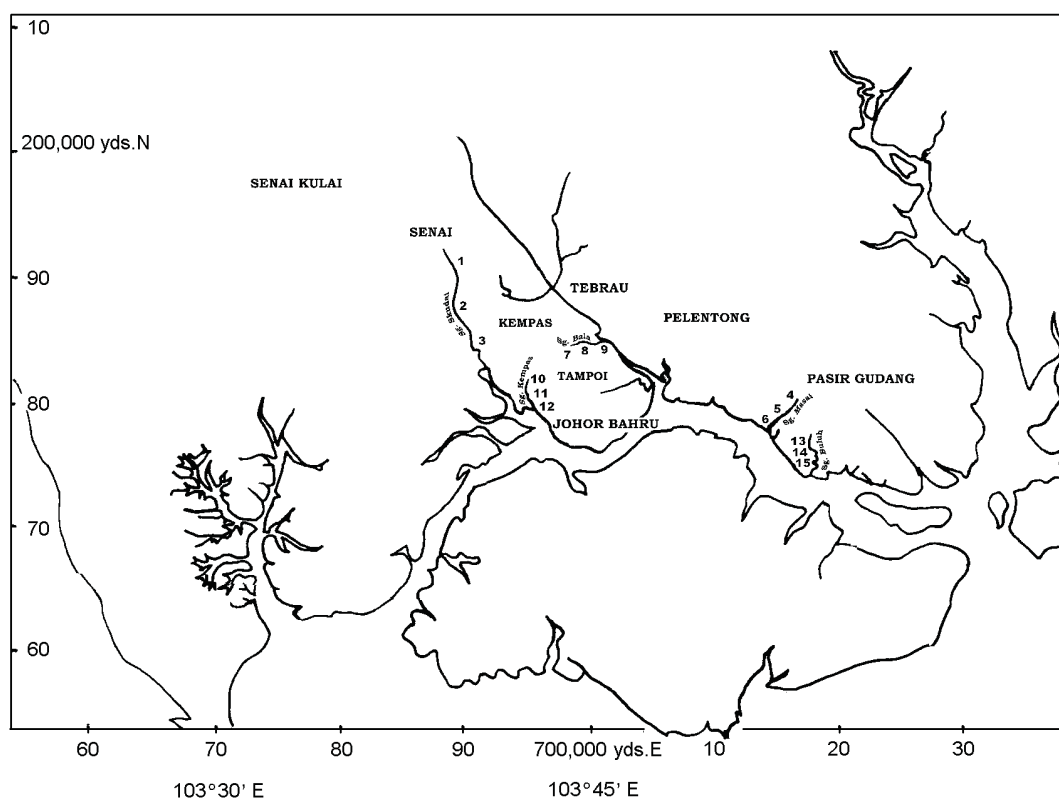


Fig. 1. Map of sampling sites in the state of Johore

Table 2. Quality of river water in the sampling sites

Location	pH	Conductivity, mS·cm ⁻¹	Dissolved oxygen, mg·l ⁻¹	Temperature, °C	Salinity, %
1	5.8	0.1	6.5	29	0
2	7.7	0.07	6.5	27	0
3	6.4	0.71	5.5	28	0
4	7.0	2.3	6.2	27	0.1
5	7.3	25.0	6.2	28	1.5
6	5.9	30.0	5.8	29	1.8
7	6.3	0.27	5.7	30	0
8	6.5	0.24	5.8	29	0
9	8.0	2.3	6.6	27	0.1
10	6.9	0.13	5.8	30	0
11	7.2	1.1	5.4	32	0
12	7.4	6.8	5.5	30	0.4
13	8.3	0.12	5.9	27	0
14	7.5	1.1	6.3	27	0
15	6.8	31.0	5.5	29	1.9

Table 3. Percentage of Cr(III) and Cr(VI) retention on Chelex-100 and accuracy of ICP-MS and INAA

Sample	Species	Retention on Chelex-100, %	
		ICP-MS	INAA
Standard solution	Cr(III)	95.84 ± 4.30	97.26 ± 1.25
Standard solution	Cr(VI)	4.61 ± 0.10	5.23 ± 0.26
CASS-2 (certified) recovery, %	Cr _(total)	108	83.69

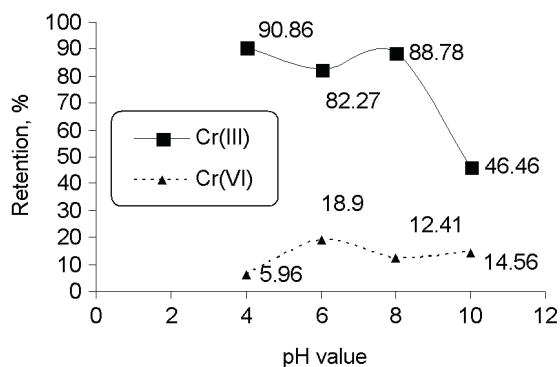


Fig. 2. The effect of pH on the retention of Cr(III) and Cr(VI) to the resin (this graph was plotted using data obtained from ICP-MS)

This speciation technique has offered satisfactory results with good reproducibility. This is evident from the results shown in Table 3 obtained from the retention rate of certain particular species on Chelex-100 using standard solutions containing Cr(III) or Cr(VI) under the optimized conditions which has been previously investigated. Since ICP-MS and INAA are unable to differentiate between Cr(III) and Cr(VI), therefore this study was carried out without mixing the standard solutions. A standard reference material (SRM) CASS-2 was used to determine the precision and accuracy of the ICP-MS and INAA analysis.

Information on the detection limit (DL) of the instrument must be confirmed before the real sample analysis was carried out. In this study, the detection limit of ICP-MS and INAA was calculated as follows:¹⁷ $DL = 3S$, where S means the standard deviation of blank solution estimate from replicate analyses. Ten replicates were used in the DL measurement and the detection limit of Cr for ICP-MS and INAA obtained were $0.033 \pm 0.01 \mu\text{g}\cdot\text{l}^{-1}$ and $0.047 \pm 0.09 \mu\text{g}\cdot\text{l}^{-1}$, respectively. The DL of Cr obtained in this study shows the availability of these instruments to detect Cr at very low concentration levels.

ICP-MS was used to determine the quantity of total Cr and Cr(VI) in the water samples. The concentration of Cr(III) was obtained by subtracting the concentration of Cr(VI) from the total Cr concentration. The concentration of Cr(III), Cr(VI) and total inorganic chromium species expressed in $\mu\text{g}\cdot\text{l}^{-1}$ or ppb in surface river water samples using ICP-MS are given in Table 4. The symbol ND used in this paper denotes non-detection of Cr species. The resin which retained the Cr(III) species was kept for INAA analysis and the results are shown in Table 5. The determination and quantification of these species in water sample is important to ensure that the treatment of the effluents is efficient to avoid large amounts of chromium containing compounds being discharged to the environment.

Table 4. Concentrations of Cr(III), Cr(VI) and total Cr in surface river waters

Location	Cr(III), $\mu\text{g}\cdot\text{l}^{-1}$	Cr(VI), $\mu\text{g}\cdot\text{l}^{-1}$	Total Cr, $\mu\text{g}\cdot\text{l}^{-1}$	pH
1	4.69 ± 0.18	2.24 ± 0.16	6.93 ± 0.07	5.8
2	1.24 ± 0.16	2.48 ± 0.16	3.71 ± 0.04	7.7
3	nd	2.90 ± 0.17	2.90 ± 0.07	6.4
4	11.35 ± 0.30	11.13 ± 0.24	22.48 ± 0.18	7.0
5	1.97 ± 0.17	3.45 ± 0.16	5.42 ± 0.08	7.3
6	48.44 ± 0.60	3.83 ± 0.19	52.27 ± 0.57	5.9
7	3.07 ± 0.19	2.54 ± 0.18	5.61 ± 0.06	6.3
8	50.06 ± 0.69	10.47 ± 0.16	60.53 ± 0.67	6.5
9	0.82 ± 0.17	2.32 ± 0.17	3.15 ± 0.02	8.0
10	0.59 ± 0.12	5.89 ± 0.12	6.48 ± 0.03	6.9
11	nd	4.23 ± 0.18	4.23 ± 0.07	7.2
12	0.14 ± 0.13	3.31 ± 0.13	3.45 ± 0.02	7.4
13	0.45 ± 0.18	3.48 ± 0.18	3.93 ± 0.04	8.3
14	nd	3.19 ± 0.16	3.19 ± 0.02	7.5
15	10.50 ± 0.28	4.88 ± 0.16	15.37 ± 0.23	6.8

nd: Not detected.

The pH of surface water plays an important role in the formation of the Cr species. The pH of these water samples are all in the range of 5.8 to 8.3 which is the normal pH range in natural waters. In aqueous solutions, Cr(III) is predicted as the stable species at $\text{pH} \leq 6$, whereas Cr(VI) in the form of CrO_4^{2-} should be predominant at $\text{pH} \geq 7$.¹⁹ The concentration of both species is dependent on to the concentration of oxygen in the water as the pH is in the range of 6 to 7.

From the observation, the Cr(VI) is referred to as the dominant species although there are no significant differences between the Cr(III) and Cr(VI) species. This is due to the high tendency of Cr(III) to form hexacoordinate octahedral complexes with a variety of ligands such as ammonia, water and urea especially when the content of humic, amino and fulvic acid in the river water is high. These Cr(III) complexes are immobile and can be removed from the river water by reducing the presence of Cr(III) species in the river. The exception of this phenomenon is observed for samples at location 6 and 8 whereby the high concentration of total chromium is due to the high concentration of Cr(III) species. Theoretically, Cr(VI) should be the dominant species in the surface water due to the dissolving of oxygen from the atmosphere inducing the oxidation of Cr(III) under acid condition. However, the nature and concentration of reducers, oxidation mediators and complexing agents play an important role such as the reduction of Cr(VI) due to the presence of Fe(II), H_2O_2 and dissolve organic matter.

Intercomparison of Cr(III) concentration obtained by ICP-MS and INAA method were performed to ensure the compatibility of both techniques. The comparison was carried out by using the Z-scores method as described by AKHTER et al.²⁰ The results obtained by both techniques are reliable if the Z-scores are between -2 to +2. Table 6 shows the results of the Z-scores

obtained for all water samples, showing that ICP-MS results are reliable in this study.

Table 5. Determination of Cr(III) concentration in surface river waters using INAA

Location	Cr(III), $\mu\text{g}\cdot\text{l}^{-1}$
1	3.10 ± 1.89
2	2.10 ± 1.21
3	nd
4	11.05 ± 2.89
5	2.01 ± 1.53
6	46.48 ± 1.63
7	4.69 ± 0.95
8	52.28 ± 0.91
9	1.47 ± 2.79
10	1.00 ± 1.53
11	nd
12	nd
13	0.24 ± 1.53
14	nd
15	8.98 ± 0.16

nd: Not detected.

Table 6. Intercomparison of INAA and ICP-MS results for Cr(III) determination

Location	Cr(III), $\mu\text{g}\cdot\text{l}^{-1}$ INAA	Cr(III), $\mu\text{g}\cdot\text{l}^{-1}$ ICP-MS	Z-score
1	3.10 ± 0.89	4.69 ± 0.18	0.84
2	2.10 ± 0.21	1.24 ± 0.16	-1.52
3	nd	nd	0
4	11.05 ± 1.89	11.35 ± 0.30	0.10
5	2.01 ± 0.53	1.97 ± 0.17	0.02
6	46.48 ± 1.63	48.44 ± 0.60	1.13
7	4.69 ± 0.95	3.07 ± 0.19	-1.67
8	52.28 ± 0.91	50.06 ± 0.69	-1.94
9	1.47 ± 0.19	0.82 ± 0.17	-0.23
10	1.00 ± 0.13	0.59 ± 0.12	-0.27
11	nd	nd	0
12	nd	0.14 ± 0.13	0.08
13	0.24 ± 0.03	0.45 ± 0.18	0.14
14	nd	nd	0
15	8.98 ± 0.16	10.50 ± 0.28	4.73

Conclusions

The presence and concentration of Cr species in water samples from the effluents depend mainly on the Cr compounds used in the industrial processes. Usually, hexavalent Cr will be dominant in wastewater from industries such as metal finishing, pigment production, and metallurgy. Cr(III) will be found mainly in tannery and textile industry wastewater. According to the Environmental Quality (Sewage and Industrial Effluents) Regulations, 1978, the maximum permissible level for Cr(III) and Cr(VI) in Standard A are $0.20 \mu\text{g}\cdot\text{ml}^{-1}$ and $0.05 \mu\text{g}\cdot\text{ml}^{-1}$, respectively.²¹ Therefore, the concentrations of the Cr species at all sampling sites did not exceed the maximum permissible level. However, the environmental monitoring for rivers must be focused to avoid any significant impacts occurring in our ecology especially by these species due to its carcinogenic properties.

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