A Robust Method for the Determination of Cr(VI) and Cr(III) in Industrial Wastewaters by Liquid Chromatography-Inductively Coupled Plasma Mass Spectrometry Combined with a Chelating Pretreatment with 2,6-Pyridinedicarboxylic Acid

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We have developed a method for the determination of Cr(VI) and Cr(III) in industrial wastewater by liquid chromatography-inductively coupled plasma mass spectrometry (LC-ICP-MS) combined with a chelating pretreatment with 2,6-pyridinedicarboxylic acid (PDCA). The PDCA unified the chemical forms of the Cr(III) species in water samples by the formation of a stable Cr(III)-PDCA complex, which was then separated by a LC column. The chromatographic mobile phase at neutral pH and the column of a mixed-bed of anion and cation exchangers successfully separated not only the chromium species without any redox conversion, but also chloride, which interfered with ICP-MS detection. The method detection limits measured at m/z 53 were $0.66 \mu\text{g of Cr L}^{-1}$ for Cr(III) and $0.74 \mu\text{g L}^{-1}$ for Cr(VI) with a sample injection volume of $20 \mu\text{L}$ under a no gas mode. The recoveries of spiked Cr(VI) at 50 and 500 g L^{-1} into the fifteen kinds of industrial wastewater samples were satisfactory ($>90\%$). The proposed method for the determination of Cr(VI) was also validated by comparing with a colorimetric method using 1,5-diphenylcarbazide prescribed by the ISO 11083 and the JIS K0102.

Keywords Chromium, speciation, liquid chromatography, inductively coupled plasma mass spectrometry, wastewater

(Received January 11, 2018; Accepted April 11, 2018; Published August 10, 2018)

Introduction

Chromium is used in large quantities in various industries, *e.g.*, steel, metal plating, chemical, and nutritional food engineering. Chromium exists in two oxidation states of trivalent chromium [Cr(III)] and hexavalent chromium [Cr(VI)] in industrial wastewaters. Cr(III) is only toxic at high concentrations, or even essential to some aquatic organisms and humans at trace levels, but Cr(VI) is a strong oxidizer and is considered highly toxic at the $\mu\text{g L}^{-1}$ level.^{1,2} Therefore, a robust and simple method to determine individual chromium species, especially Cr(VI), is required for the risk assessment of chromium in industrial wastewater.

Individual determinations of Cr(III) and Cr(VI) require the preservation of original oxidation states of Cr(III) and Cr(VI) during analysis. However, the oxidation states of the chromium species change in some cases because of the dependence of the pH and coexisting substances in sample water. For example, Cr(III) is oxidized to Cr(VI) at alkaline pH, while Cr(VI) is

reduced to Cr(III) at acidic pH, especially in the presence of organic substances.^{3,4} It also requires one to unify various chemical forms of Cr(III) species for separation from Cr(VI), because Cr(III) can form complexes with coexisting substances and water molecules as ligands in industrial wastewater samples, *e.g.*, $\text{Cr}(\text{H}_2\text{O})_6^{3+}$ and $\text{Cr}(\text{acetate})_3$. Because of difficulties, a few methods for Cr speciation were investigated to apply to high-matrix samples, *e.g.*, industrial wastewaters, while many methods in low-matrix water samples, *e.g.*, stream water, tap water,⁵ and ground water,⁶ were previously reported.

A colorimetric method using 1,5-diphenylcarbazide (DPC) for the determination of Cr(VI) in industrial waste water is prescribed as a standard method by the International Organization for Standardization (ISO) 11083⁷ and the Japanese Industrial Standards (JIS) K0102.⁸ However, the method is complicated, directly inapplicable to Cr(III), and seriously interfered by colored coexisting substances, *e.g.*, iron. To determine Cr(III) and Cr(VI) simply and simultaneously, an online method based on liquid chromatography combined with the pre-column coloration of Cr(III) using 2,6-pyridinedicarboxylic acid (PDCA) and the post-column coloration of Cr(VI) using DPC was reported.⁹ However, this method still suffered from interference by coexisting colored substances and the detection limit for Cr(III) were also high, *e.g.*, $30 \mu\text{g L}^{-1}$, even with a large sample injection volume ($250 \mu\text{L}$). To overcome the interference and to improve the detection limit, inductively

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coupled plasma mass spectrometry (ICP-MS),^{5,10} inductively coupled plasma optical emission spectroscopy (ICP-OES),¹⁰ and atomic adsorption spectrometry (AAS)¹¹ were online coupled with liquid chromatography (LC). The detection methods were also combined with off-line separation and/or preconcentration techniques, such as solid-phase extractions,¹²⁻¹⁴ solvent extraction,¹⁵ and cloud-point extraction.¹⁶ However, off-line coupling is operationally complicated for routine analysis and less-quantitative due to manual sample manipulations in some cases.¹³

Among the methods for Cr speciation, the on-line coupling of LC and ICP-MS (LC-ICP-MS) is considered to be a greater advantage for owing to simplicity, sensitivity, and selectivity. The methods are classified into two groups based on the use or non-use of a chelating pretreatment to unify the chemical forms of Cr(III) species in water samples by forming a stable Cr(III) complex. Although LC-ICP-MS without any pretreatment has the advantage of being able to use diluted nitric acid as a mobile phase that is suitable for ICP-MS detection,⁵ but it has a serious drawback in that the multiple peaks of Cr(III) are detected because Cr(III) exists as multiple chemical forms in the presence of coexisting substances in real water samples. For example, three peaks were reported in the presence of hydrogencarbonate ions.⁵ Because of this reason, the LC-ICP-MS methods without the pretreatment have not been validated to industrial wastewater samples containing large amounts of coexisting substances,⁴ and applied only to low-matrix water samples¹⁰ *e.g.*, stream and lyophilized water.

On the other hand, LC-ICP-MS with the chelating pretreatment has the advantage of being able to overcome problems caused by multiple peaks. Ethylenediaminetetraacetic acid (EDTA) has been used as a chelating agent to form a stable Cr(III)-EDTA complex.^{6,10,17} The Cr(III)-EDTA complex and Cr(VI) were separated by anion-exchange chromatography using a mobile phase that contained *e.g.*, EDTA, oxalic acid and ammonia at pH 7,⁶ sulfate at pH 9,^{10,17} or a combination of nitrate and phosphate at pH 7.¹⁷ These methods were applied to tap water and groundwater⁶ and one kind of industrial wastewater, but with little information about the coexisting substances.¹⁷ The use of such nonvolatile inorganic salts at a high concentration (over 10 mmol L⁻¹ level) in the mobile phase^{10,17} and at higher flow rates (over 1.0 mL/min) interfered with the long-term stability of ICP-MS detection due to salt deposition on the sampling cone of an ICP-MS instrument. By introducing an ion chromatographic suppressor before ICP-MS detection, the inorganic salts were exchanged to the corresponding acids, *e.g.*, nitric acid, which reduced the salt deposition. This technique made it possible to apply to a diluted estuarine water.¹⁸ However, a large amount of chloride or HCl generated by an exchange of chloride by the suppressor still interfered with the measurement of Cr(III) at *m/z* 52 and 53 owing to molecular ions, such as ³⁵Cl¹⁶O¹H⁺ and ³⁷Cl¹⁶O⁺, respectively, because the peak of the chloride was very close to that of the Cr(III)-EDTA complex.

The purpose of this study was to develop a robust and simple method for the separate determinations of Cr(III) and Cr(VI) in industrial wastewater samples by LC-ICP-MS with a chelating pretreatment. The principle of the separation was adapted from a technical note⁹ in which an LC-colorimetric method was combined with a chelating pretreatment of Cr(III) using 2,6-pyridinedicarboxylic acid (PDCA). This method is expected to unify the chemical forms of the Cr(III) species and to preserve the original oxidation states of Cr(III) and Cr(VI) during analysis because of the neutral pH conditions for the chelating pretreatment and LC separation. The chromatographic column of a mixed-bed of anion and cation exchangers is expected to

Table 1 Types of industrial wastewater samples

No.	Sample
1	Metal plating factory 1
2	Metal plating factory 2
3	Semiconductor factory 1
4	Biological treatment facility of No. 3
5	Leachate treatment facility of industrial wastes
6	Paper mill
7	Filtration treatment facility of No. 6
8	Food factory 1
9	Biological treatment facility of No. 8
10	Dyehouse
11	Pharmaceutical factory
12	Biological treatment facility of food factory 2
13	Semiconductor factory 2
14	Biological treatment facility of No. 13
15	Desulfurization treatment facility of power plant

separate not only chromium species, but also interfering substances, like chloride. We optimized the conditions of the chelating pretreatment and LC separation suitable for coupling to ICP-MS detection with long-term stability. The interferences on the analysis and redox conversion of chromium from coexisting substances were extensively investigated, and the applicability of the proposed method was examined using fifteen kinds of real industrial wastewater samples collected from eight industrial sectors. The validation of the proposed method for the determination of Cr(VI) was also carried out by comparing with a standard colorimetric method using DPC.

Experimental

Reagents and chemicals

All solutions including mobile phases were prepared with ultrapure water (18 MΩ cm) obtained from a Millipore Milli-Q water purification system (Bedford, MA, USA). Working solutions of Cr(III) and Cr(VI) were prepared by diluting the 1000 mg L⁻¹ Cr(III) and Cr(VI) stock solutions purchased from SPEX CertiPrep (Metuchen, NJ, USA), respectively. A stock solution of 2,6-pyridinedicarboxylic acid (PDCA) for the chelating pretreatment and a mobile phase contained 20 mmol L⁻¹ PDCA (≥99.5%, Sigma-Aldrich, St. Louis, MO, USA), 20 mmol L⁻¹ sodium phosphate dibasic anhydrous (≥99.5%, pharma grade, Sigma-Aldrich), and 500 mmol L⁻¹ acetic acid (ultrapure reagent, Kanto Chemical). The solution was adjusted to pH 6.8 by adding an ammonia solution (ultrapure reagent, Kanto Chemical) prior to filling-up to the final constant volume. The solution for the chelating pretreatment was used by diluting 10-times the stock solution. The mobile phase for LC was prepared by diluting 10-times the stock solution and dissolving sodium iodide (>99.99%, Suprapur®, Merck KGaA, Darmstadt, Germany) to the diluted solution. The final concentration of sodium iodide was 10 mmol L⁻¹. All of the solutions were stored in polyethylene bottles at 4°C before use. The other reagents used in this study were commercially available and of analytical-grade.

Preparation of industrial wastewater samples

Fifteen kinds of industrial wastewater samples were collected from eight industrial sectors, as listed in Table 1. All samples were filtered through 0.45-μm cellulose nitrate membrane filters (Advantec Toyo Kaisha, Tokyo, Japan). The filtrates were

transferred into polyethylene bottles with a fluoropolymer-lined cap and stored in the dark at 4°C until analysis.

Chelating pretreatment

A portion of 2 mL of an industrial wastewater sample was transferred into a 50-mL polypropylene tube. A 2-mL volume of the PDCA stock solution (pH 6.8) and 10 mL of water were added. The pH of the solution was adjusted to $\text{pH } 6.8 \pm 0.1$ with a diluted ammonia solution, if necessary. Finally, water was added up to 20 mL. The water sample was 10-times diluted. In the case of sample solutions of Cr standard or for examining the influence of any coexisting substance, a sample solution for a chelating pretreatment was prepared by adding 1 mL of a stock standard solution of Cr(III) or Cr(VI) to 8 mL of water or an aqueous solution containing a model substance, and finally adding 1 mL of the PDCA stock solution. A portion of 1 mL of the prepared solution was transferred into a vial and heated on a water bath for 30 min at 80°C for chelating Cr(III) with PDCA. The prepared solutions were analyzed within 6 h.

Instrumentation

LC separation. The separation of Cr(III) and Cr(VI) was performed using an IonPac CG5A guard column (9 μm particle diameter, 4 mm i.d. $\times$ 50 mm, Dionex Corporation, Sunnyvale, CA, USA). The column has cation and anion exchange capacities because of the presence of sulfonic acid and alkanol quaternary ammonium functional groups on a divinylbenzene/styrene polymer and a polyvinylbenzylammonium polymer, respectively. The mobile phase, as shown in Table 2, was delivered at a flow rate of 0.2 mL min⁻¹ with a metal-free high-performance liquid-chromatography pump (intelligent pump 301, FLOM Corporation, Tokyo, Japan). Samples were injected into the columns by means of a 20 or 100 μL PTFE injection loop, attached to a metal-free PEEK injection valve (C2-1346, VICI Valco Instruments, Houston, TX, USA). The outlet of the HPLC column was directly connected to a nebulizer of ICP-MS via 20-cm PTFE tubing (0.17 mm i.d.).

ICP-MS. Chromium in the prepared solution was determined with an ICP-MS instrument (Agilent 7700x, Agilent Technologies, Tokyo, Japan). A concentric nebulizer and a double-pass spray chamber were used. The ICP-MS operating parameters are listed in Table 2.

Results and Discussion

Optimization of conditions of the LC and chelating pretreatment

In order to develop a method for the separate determination of Cr(VI) and Cr(III) by LC-ICP-MS with a chelating pretreatment, we firstly adopted a method that was previously reported for an LC-spectrometric method using 1,5-diphenylcarbazide.¹ The solution initially used for the chelating pretreatment and for the mobile phase of LC contained 2 mmol L⁻¹ PDCA, 2 mmol L⁻¹ Na₂HPO₄, 10 mmol L⁻¹ NaI, 50 mmol L⁻¹ CH₃COOH, 2.8 mmol L⁻¹ LiOH. The solution was also adjusted at pH 6.8 with a diluted NaOH solution. The initial flow rate of the mobile phase was 1.0 mL min⁻¹. However, such a high flow rate and high concentrations of the non-volatile and refractory salts in the mobile phase seriously interfered with the ICP-MS detection, and caused salt deposition at the nebulizer tip and the ICP-MS interface. To reduce the total amount of non-volatile salts, the necessity of each component in the mobile phase and chelation and the reduction of flow rate of the mobile phase were examined. While it is considered that lithium ion works to elute ionic species, it is well known that lithium causes a deposition

Table 2 Optimized conditions of the proposed method

Chelating pretreatment	
Pretreatment solution	2 mmol L ⁻¹ PDCA, 2 mmol L ⁻¹ Na ₂ HPO ₄ , 50 mmol L ⁻¹ CH ₃ COONH ₄ , pH 6.8
LC	
Column	IonPac CG5A, 4 $\times$ 50 mm (Dionex Corporation, Sunnyvale, CA, USA)
Mobile phase composition	2 mmol L ⁻¹ PDCA, 2 mmol L ⁻¹ Na ₂ HPO ₄ , 10 mmol L ⁻¹ NaI, 50 mmol L ⁻¹ CH ₃ COONH ₄ , pH 6.8
Separation temperature	Room temperature
Injection volume	20 or 100 μL
Flow rate	0.2 mL min ⁻¹
ICP-MS	
Instrument	Agilent 7700x
Nebulizer	Quartz concentric
RF power	1550 W
Plasma gas flow rate	15 L min ⁻¹
Carrier gas flow rate	1.0 L min ⁻¹
Make-up gas flow rate	0.2 L min ⁻¹
Monitored isotope	⁵³ Cr (without collision mode) ⁵² Cr (with collision cell H ₂ mode, H ₂ 4.0 mL min ⁻¹)

on the sampling cone of the ICP-MS instrument as well as severe memory effects in ICP-MS.¹⁹ We evaluated the separation efficiency of Cr(VI) and Cr(III) with and without lithium ion, and found no significant difference between them. Thereby, lithium was excluded from the mobile phase. Sodium hydroxide used for the pH adjustment was also excluded and an ammonia aqueous solution was used instead of NaOH.

The flow rate of the mobile phase should be decreased to reduce the salt amounts introduced into the ICP-MS instrument. For this purpose, we adopted a short length column (Dionex CG5A, 50 mm length) instead of a 250 mm-long column (Dionex CS5A), although CG5A is usually used as a guard column. Because of this miniaturization, the flow rate could be reduced from 1.0 to 0.2 mL min⁻¹, which is calculated as the bed height divided by the linear velocity. The chromatogram obtained at 10 $\mu\text{g L}^{-1}$ Cr(VI) is shown in Fig. S1. The sensitivity is defined as the signal height (cps) divided by the concentration ($\mu\text{g L}^{-1}$) and the instrumental detection limit is calculated from 3-times the standard deviation (SD) of three replicates analyses of the blank divided by the sensitivity. The sensitivity with the 50 mm-long column (695) was 1.6-times higher than that of a 250 mm-long column (443), and the instrumental detection limit with the former column (0.38 $\mu\text{g L}^{-1}$) was 1.9-times lower than that with the latter column (0.71 $\mu\text{g L}^{-1}$). The reason why a better sensitivity and a lower detection limit were obtained at a lower flow rate (sample uptake rate into ICP-MS) in spite of the fact that the same absolute amounts of Cr(VI) was introduced into the column is considered to be that the dilution of Cr(VI) with the mobile phase was suppressed, the nebulization efficiency was improved, and ionization depression owing to overloading by the solvent in the plasma was reduced. The SD of the blank was also reduced because of less overloading, which contributed to the lower detection limit. Thus, a 50 mm-long column and a low flow rate (0.2 mL min⁻¹) were adopted in the following experiments.

As for the chelating pretreatment, we found that both the lithium and iodide salts could be removed without any deterioration of the separation and recoveries of Cr(VI) and

Table 3 Effect of lithium hydroxide and sodium iodide on the recovery of chelating pretreatment

LiOH	NaI	Relative detection intensity, %	
		Cr(III)	Cr(VI)
+	+	100 ^a	100 ^a
–	–	99.8 (4.7)	101 (4)

a. Recovery value obtained with both LiOH and NaI was normalized to 100.

Figures are recovery percentage and the figures in bracket are standard deviation of two replicate tests.

Fig. 1 Chromatograms obtained from (a) No. 12 wastewater and samples spiked with (b) Cr(III) and (c) Cr(VI) at $500 \mu\text{g L}^{-1}$. Injection volume, $20 \mu\text{L}$. ICP-MS measurements were carried out without the collision cell mode. The wastewater sample was 10-times diluted at analysis by LC-ICP-MS. The chromatograms were elevated by (b) 5000 and (c) 10000 cps, respectively, for better visual observation.

Cr(III), as shown in Table 3. The recovery result for each chromium species in the absence of both lithium and iodide salts was satisfactory (*ca.* 100%). The optimized conditions of the LC separation and the chelating pretreatment are summarized in Table 2; the chromatograms obtained with these conditions are shown in Fig. 1 and Fig. S2.

Figures of merit

The method detection limits (MDLs) were evaluated as follows. A test solution was prepared by spiking each standard of Cr(III) or Cr(VI) to give the final concentration at $1 \mu\text{g L}^{-1}$ in a 10-fold diluted solution of the PDCA stock solution at the chelating pretreatment. Three replicates were analyzed by the proposed method, including the chelating pretreatment and LC-ICP-MS measurements without the collision cell mode. The sample injection volume into LC-ICP-MS was $20 \mu\text{L}$. The MDLs for Cr(III) and Cr(VI) were calculated using a value of 3-times the standard deviation (SD)/sensitivity, which is defined as the signal peak area divided by the concentration. The MDLs for Cr(III) and Cr(VI) at $20 \mu\text{L}$ injection at m/z 53 were 0.66 and $0.74 \mu\text{g L}^{-1}$, respectively.

In contrast, with the collision cell mode, the MDLs for Cr(III) and Cr(VI) at $20 \mu\text{L}$ injection were lowered to 0.092 and $0.077 \mu\text{g L}^{-1}$, respectively, at m/z 52, as shown in Fig. S2(a).

Increasing the injection volume of samples to $100 \mu\text{L}$ further improved the MDLs for Cr(III) and Cr(VI) at m/z 52: 0.023 and $0.047 \mu\text{g L}^{-1}$, respectively. The chromatograms are shown in Fig. S2(b). However, the decrease in the ICP-MS intensity, probably due to water in the sample solution, was observed prior to the peak of Cr(III)-PDCA, which possibly influenced the determination of Cr(III), especially trace levels (sub ppb).

These MDLs for Cr(VI) with/without the collision cell mode were much lower than the environmental quality standard for water pollution in Japan (EQSJ, $50 \mu\text{g L}^{-1}$) and the effluent standard in Japan (ESJ, $500 \mu\text{g L}^{-1}$). These MDLs were also two orders of magnitude lower than that obtained with spectrometric method using 1,5-diphenylcarbazide (current standard method of Japan, JIS K 0102 65.2.1). The calibration curves for Cr(III) and Cr(VI) in the range from 1 to $100 \mu\text{g Cr L}^{-1}$, respectively, have high linearity; the correlation coefficients (*R*) for Cr(III) and Cr(VI) were 0.9999 and 0.9997, respectively. We evaluated the speciation purities of chromium in the standard solution of Cr(VI) and Cr(III) by each calibration of the individual Cr species. Only a trace amount of Cr(III) was found as impurities in the standard solutions of Cr(VI). The determined content was 1.2% using a standard solution of Cr(III), which indicated no significant influence on the prepared concentration of Cr(VI) and the determinations of Cr(VI) and Cr(III) in samples.

Influence of coexisting substances

Coexisting substances in industrial wastewater samples are liable to interfere with the quantification of Cr(III) and Cr(VI) in the stages of the chelating pretreatment, LC separation and ICP-MS detection. The influence from coexisting substances was evaluated using recovery tests in which each standard of Cr(III) or Cr(VI) was spiked to give the final concentration at $50 \mu\text{g L}^{-1}$ in an aqueous solution containing a coexisting substance at the chelating pretreatment. The kinds of the inorganic coexisting substances were basically set to those that were found in the real wastewater samples, as shown in Table S1. The concentrations of the coexisting substances were also set to those in the real samples after 10-fold dilution because of the PDCA pretreatment. The organic coexisting substances that can also interfere with the complexation of Cr(III) with PDCA, *i.e.*, organic acids and EDTA, were examined because organic substances certainly exist from the TOC measurement data in Table S1. LC-ICP-MS measurements were performed without the collision cell and by using a $20\text{-}\mu\text{L}$ injection volume. The conditions were adopted in the following experiments. The results for Cr(III) and Cr(VI) are given in Tables 4 and 5, respectively.

Among the inorganic cations that are commonly found in industrial wastewater samples, Fe^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Ca^{2+} and Mg^{2+} may inhibit the complexation of Cr(III)-PDCA by competitive binding to PDCA. In practice, no interferences from these cations were observed for the complexation of Cr(III)-PDCA, oxidative conversion to Cr(VI), and subsequent quantification of Cr(III) in ranges up to 10 mg L^{-1} of the transition metals and up to 100 mg L^{-1} of the alkaline earth metals. However, significant interferences were observed at concentrations of Co^{2+} and Ni^{2+} that are more than 100 mg L^{-1} . Since the oxidative conversion to Cr(VI) was not observed, it is considered that the PDCA was predominantly complexed with large amounts of the transition metals that were not used for complexation with Cr(III), or that the Cr(III) was co-precipitated with the hydroxylates of the transition metals. As for the recoveries for Cr(VI), no reductive conversion to Cr(III) was observed in the presence of Ni^{2+} and Co^{2+} , even at high

Table 4 Influence of coexisting substances on the recovery of Cr(III)^a

Coexisting substance	Concentration/mg L ⁻¹	Cr-U2 (RT1.6)	Cr(III)-PDCA	Cr-U4 (RT3.4)	Cr(VI)	Sum of Cr species
Mg	100	—	102 (0.4)	—	—	102 (0.4)
Ca	100	—	92.2 (2.3)	—	—	92.2 (2.3)
Al	1	—	102 (0.3)	—	—	102 (0.3)
Fe(II)	1	—	109 (2)	—	—	109 (2)
Fe(III)	1	—	106 (2)	—	—	106 (2)
	10	—	101 (3.0)	—	—	101 (3.0)
Co	10	—	102 (2)	—	—	102 (2)
	100	—	5.7 (0.3)	—	—	5.7 (0.3)
Ni	1	—	97.7 (1.5)	—	—	97.7 (1.5)
	10	—	98.5 (0.7)	—	—	98.5 (0.7)
	200	—	6.1 (0.4)	—	—	6.1 (0.4)
Cu	10	—	96.4 (2.5)	—	—	96.4 (2.5)
Cl	100	—	107 (1.5)	—	—	107 (1.5)
	1540	—	99.3 (2.0)	—	—	99.3 (2.0)
Nitrate	1770 (400 as N)	—	99.9 (4.8)	—	—	99.9 (4.8)
Sulfate	10	—	92.3 (0.0)	—	—	92.3 (0.0)
EDTA	10 (0.035 mmol L ⁻¹)	—	98.8 (2.2)	—	—	98.8 (2.2)
	577 (2 mmol L ⁻¹)	92.4 (3.0)	3.9 (1.6)	—	—	96.3 (4.6)
Tartaric acid	33 (10 as C)	—	98.7 (1.3)	—	—	98.7 (1.3)
	331 (100 as C)	—	102 (0.2)	0.9 (0.0)	—	103 (0.2)
Tartaric acid (after overnight)	33 (10 as C)	—	103 (2.0)	—	—	103 (2.0)
	331 (100 as C)	—	102 (2.2)	1.2 (0.6)	—	103 (2.8)
Ascorbic acid	22 (10 as C)	—	95.8 (2.7)	—	—	95.8 (2.7)

a. Figures are recovery percentage and the figures in bracket are standard deviation of two replicate tests. —; Not detected. The value was calculated using the MDL of the proposed method. RT; retention time (min).

Table 5 Influence of coexisting substances on the recovery of Cr(VI)^a

Coexisting substance	Concentration/mg L ⁻¹	Cr-U1 (RT1.4)	Cr-U3 (RT1.7)	Cr(III)-PDCA	Cr(VI)	Sum of Cr species
Mg	100	—	—	2.4 (0.2)	98.2 (0.5)	100.6 (0.7)
Ca	100	—	—	0.4 (0.1)	95.5 (1.5)	95.9 (1.6)
Al	1	—	—	3.5 (1.5)	99.5 (1.2)	103 (2.7)
Fe(II)	1	—	—	85.5 (3.2)	11.5 (0.9)	97 (4.1)
Fe(III)	1	—	—	1.5 (0.3)	103 (2.0)	105 (2.3)
	10	—	—	0.4 (0.6)	91.0 (4.5)	91.4 (5.1)
Co	100	—	—	—	100 (4)	100 (4)
Ni	200	—	—	—	103 (0.7)	103 (0.7)
Cu	10	—	—	5.8 (1.1)	90.6 (0.2)	96.4 (1.3)
Cl	100	—	—	3.9 (0.8)	97.9 (1.1)	102 (1.4)
	1540	—	—	6.7 (0.6)	91.7 (1.4)	98.4 (2)
Nitrate	1770 (200 as N)	—	—	2.6 (0.2)	94.0 (5.1)	96.6 (5.2)
Sulfate	10	—	—	3.1 (0.1)	100 (4.2)	103 (4.3)
EDTA	10 (0.035 mmol L ⁻¹)	—	—	1.4 (0.6)	102 (2.0)	103.4 (2.6)
	577 (2 mmol L ⁻¹)	1.8 (0.6)	4.0 (0.2)	—	91.2(0.2)	97 (1)
Tartaric acid	33 (10 as C)	—	—	2.4 (0.2)	96.2 (0.7)	98.6 (0.9)
	331 (100 as C)	—	—	4.6 (0.6)	91.9 (0.5)	96.5 (1.1)
Tartaric acid (after overnight)	33 (10 as C)	—	—	4.0 (0.5)	98.6 (1.9)	103 (2.1)
	331 (100 as C)	—	—	22 (0.4)	71.5 (0.2)	93.5 (0.6)
Ascorbic acid	22 (10 as C)	—	—	87.3 (1.3)	—	87.3 (1.3)

a. Figures are recovery percentage and the figures in bracket are standard deviation of two replicate tests. RT; retention time (min).

concentrations: 200 and 100 mg L⁻¹, respectively. Only a slight reductive conversion to Cr(III) was observed at 10 mg L⁻¹ of Fe³⁺, and Cu²⁺; that is, several percent of Cr(VI) was reduced to Cr(III) during the chelating pretreatment. On the other hand, the recovery at 1 mg L⁻¹ Fe²⁺ was drastically lowered to *ca.* 12%. From the fact that a newly generated peak corresponding to Cr(III)-PDCA complex was observed, and that the sum of Cr(III) and Cr(VI) was close to 100%, it is considered that Cr(VI) was reduced to Cr(III) at the stage of the chelating pretreatment and/or prior to the pretreatment because of

reduction-labile Fe²⁺.

Inorganic anions may not inhibit the complexation of Cr(III)-PDCA, but may interfere with the LC separation and ICP-MS detection, *e.g.*, ³⁷Cl¹⁶O⁺ interfered on ⁵³Cr⁺. No influence from chloride, nitrate and sulfate on the recovery for Cr(III) was observed. Since chloride (retention time, 1.4 min) was separated from a Cr(III)-PDCA complex (retention time, 2.7 min) by the LC, there was no interference even at high concentration above 1500 mg L⁻¹, as also shown in Fig. S3. For Cr(VI), only a few percent of Cr(VI) was reduced to Cr(III) during the chelating

pretreatment.

Organic substances, especially chelating agents, may inhibit the complexation of Cr(III) with PDCA by competitive binding. As a representative compound, EDTA was examined. When the concentration of EDTA ($0.035 \text{ mmol L}^{-1}$) was much lower than that of PDCA (2 mmol L^{-1}), there was no interference on the Cr(III)-PDCA complexation. Meanwhile, when the concentration of EDTA (2 mmol L^{-1}) was equivalent to that of PDCA, Cr(III) predominantly complexed not with PDCA, but with EDTA, and consequently the recovery drastically became lower. The other organic compounds, *i.e.*, tartaric acid and ascorbic acid, showed no interference for the quantification of Cr(III). No oxidative conversion of Cr(III) to Cr(VI) was also observed in the case of all of the organic compounds examined in this study.

In contrast, ascorbic acid had a very severe influence on the recovery of Cr(VI) because it rapidly reduced Cr(VI) to Cr(III). It is considered that reduction occurred at the stage of the chelating pretreatment and/or prior to the pretreatment.²⁰ It was also found that tartaric acid and EDTA slowly reduced Cr(VI) to Cr(III). For example, when tartaric acid coexisted with Cr(VI) overnight at a high concentration (331 mg L^{-1}), *ca.* 20% of Cr(VI) was reduced to Cr(III), and when EDTA coexisted with Cr(VI) at 2 mmol L^{-1} , *ca.* 6% of Cr(VI) was reduced and complexed with EDTA. It is considered that the reduction with tartaric acid mainly progressed prior to the stage of the chelating pretreatment, and that the reduction with EDTA slowly occurred at the pretreatment stage. Since Cr(VI) can be reduced to Cr(III) at higher concentrations of coexisting organic substances, even at neutral pH, the analysis of Cr(VI) should be done as soon as possible after sampling.

Hitherto, there has been no detailed information concerning the interferences on the analysis and the redox conversion of chromium due to the coexisting substances found in industrial wastewater samples. The present study revealed interferences on the analysis and redox conversion due to samples for the first time. Although chelating agents, like highly concentrated EDTA for Cr(III) and transition metals for PDCA, showed severe interferences for the formation of the Cr(III)-PDCA complex, most of the coexisting substances experienced negligible or only slight interferences. On the other hand, some of the coexisting substances in samples naturally have the possibility to undergo the redox conversion of Cr(VI) or Cr(III) prior to any chelating pretreatment, *e.g.*, the reduction of Cr(VI) to Cr(III) by Fe^{2+} or ascorbic acid in samples. We believe that the present method is robust enough to be applied to industrial wastewater samples, *i.e.*, interference-less, and that the method can minimize any redox conversion of chromium at analysis, as shown in the analysis of standard solutions of Cr species. It should be also noted that if the coexisting substances are unknown, the recovery test of individual chromium species, *i.e.*, Cr(III) and Cr(VI), and comparisons between the sum of the individual chromium species and the spiked concentration of the total chromium should be done before any analysis. It is considered that the recovery results represent both the interference on the analysis and the redox conversion due to the samples. Also, if agreement between the sum of each chromium species and the spiked total Cr is obtained, only the redox conversion of the chromium species due to samples can be observed; however, no interference on the analysis occurs. This study evaluated both the recovery tests and comparisons in examinations of the influence of coexisting substances and the applicability to real samples, as mentioned below.

Applicability to industrial wastewater samples

We evaluated the applicability of the proposed method to real industrial wastewater samples by recovery tests in which each standard of Cr(III) or Cr(VI) was spiked to give the final concentration at 50 and $500 \mu\text{g L}^{-1}$. These concentration values corresponded to EQSJ and ESJ, respectively. As shown in Table 1, fifteen kinds of samples were collected from eight industrial sectors: metal plating, semiconductor, waste treatment, paper mill, dying, pharmacy, food, and power plant. Some of the wastewater samples were treated biologically. The samples were 10-fold diluted at the stage of the chelating pretreatment with PDCA. An example of chromatographic data obtained from industrial wastewaters spiked with Cr(III) and Cr(VI) is shown in Fig. 1. The recovery results are presented in Fig. 2. To evaluate the interference on the recovery results, the coexisting substances were determined by ICP-OES, ICP-MS, a TOC analyzer, and ion chromatography, as summarized in Table S1. The recoveries of Cr(VI) at 50 and $500 \mu\text{g L}^{-1}$ were almost satisfactory (*ca.* 90–110%) for all of the fifteen wastewater samples, although reductive conversions to Cr(III) were observed in several samples, but the conversion rates were limited to below 11 and 3% at 50 and $500 \mu\text{g L}^{-1}$, respectively. Coexisting sodium, nickel, nitrate and chloride over 1000 mg L^{-1} in the wastewater samples or its biologically-treated water of a plating factory (Nos. 1 and 2), a leachate treatment facility of industrial wastes (No. 5) and a desulfurization treatment facility of a power plant (No. 11) gave no significant interferences and reductive conversion due to samples on the determination of Cr(VI).

The recoveries of Cr(III) at 50 and $500 \mu\text{g L}^{-1}$ were also satisfactory for ten wastewater samples (Nos. 3, 4, 5, 6, 7, 11, 12, 13, 14 and 15), *e.g.*, a semiconductor, waste treatment, a paper mill, a pharmacy, food, and power plant. No significant interference and oxidative conversion due to samples were observed, even when sodium, chloride and sulfate, respectively, coexisted at over 1000 mg L^{-1} and magnesium potassium, calcium, cadmium, nitrite and TOC, respectively, coexisted at over 100 mg L^{-1} . For example, the results for No. 12, as shown in Figs. 1 and 2(b), indicated that most of the Cr(III) (>90%) was detected as the PDCA complex without any oxidative conversion to Cr(VI), while the other kinds of Cr(III) species that probably bound the other coexisting substances were slightly (a few percent) detected at retention times of around 1.5–2.5 min. However, the spiked Cr(III) was not recovered at all from the wastewaters and its biologically-treated water of a plating factory (Nos. 1 and 2). This is because the PDCA was predominantly complexed with large amounts of transition metals (nickel, cadmium, and cobalt) at over 1000 mg L^{-1} , and was not used for complexation with Cr(III). Another reason is considered to be that the co-precipitation of Cr(III) with the hydroxylation reaction of transition metals by increasing the pH of the acidic sample solutions occurred. The recoveries of Cr(III) at 50 and 500 g L^{-1} from food factory wastewater (No. 8) were *ca.* 80 and 70%, respectively. Considering that the TOC concentration was relatively high, and that unidentified chromium species (Cr-U1 and Cr-U2) were observed at 500 g L^{-1} , a possible reason for the low recoveries is that the coexisting organic substances strongly bound Cr(III) and inhibited complexation with PDCA. Similar tendencies were observed in biologically treated water (No. 9) of the food factory. The recoveries of Cr(III) at 50 and 500 g L^{-1} from a dye house wastewater (No. 10) were also *ca.* 80 and 70%, respectively. In contrast, the unidentified chromium species (Cr-U1 and Cr-U2) that were formed by complexes with non-PDCA ligands were not observed even though the TOC

Fig. 2 Recovery results for fifteen industrial wastewater samples: (a) Cr(III) at $50 \mu\text{g L}^{-1}$, (b) Cr(III) at $500 \mu\text{g L}^{-1}$, (c) Cr(VI) at $50 \mu\text{g L}^{-1}$ and (d) Cr(VI) at $500 \mu\text{g L}^{-1}$. DPC: determined by colorimetric method using 1,5-diphenylcarbazide (DPC).

concentration was very high. Another reason is considered to be that the Cr(III) was precipitated after spiking into the highly alkaline sample solution.

Comparison with a spectrometric method using 1,5-diphenylcarbazide

A validation of the proposed method for the determination of Cr(VI) was carried out by comparing the recovery rate with a standard method: a spectrometric method using 1,5-diphenylcarbazide (DPC), prescribed in JIS K0102 65.2.1. The comparison data for four kinds of industrial wastewaters (Nos. 12 – 15) are shown in Figs. 2(c) and 2(d). The present method and spectrometric method gave comparable results for a semiconductor factory wastewater and its biologically treated water, and the desulfurized wastewater of a power plant (Nos. 13 – 15). In contrast, in the case of the biologically treated water of a food factory (No. 12), the present method gave a recovery of almost 100%, while the spectrometric method gave only 47 and 68% at 50 and 500 g L^{-1} , respectively. These results and the results of measurements of the coexisting substances suggested that the spiked Cr(VI) was partly reduced to Cr(III) with coexisting organic substances during operation of the spectrometric method.

In conclusion, we demonstrated that the proposed method was widely applicable to separate determinations of Cr(III) and Cr(VI) in various industrial wastewater samples. We also clarified the limitation of this method for samples containing large amounts of coexisting substances, and the alternation of the oxidation states of the chromium species spiked into the

sample by redox conversion due to the substances. For example in the former case, transition metals such as Ni^{2+} and Co^{2+} or organic chelating substances like EDTA interfered with the determination of Cr(III) owing to an inhibition of the complexation of Cr(III)-PDCA. In the latter case, reducing substances such as ascorbic acid and Fe^{2+} interfered the determination of Cr(VI) by reductive conversion to Cr(III). The method was also validated by comparing with a spectrometric method (JIS K 0102), and a fairly superior performance to the current standard method was obtained in the cases of a food factory wastewater. The proposed method was robust to coexisting substances and the operation was simple and rapid. These characteristics are suitable for the analysis of industrial wastewater samples.

Acknowledgements

We are grateful to Ms. Setsuko Iso (Organo Corporation, Tokyo, Japan) for providing the industrial wastewater samples.

Supporting Information

The supporting information (Table S1, Figs. S1 – S3) is available free of charge on the web at <http://www.jsac.or.jp/analsci/>.

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