Determination of chromium(III), chromium(VI) and total chromium in chromate and trivalent chromium conversion coatings by electrospray ionization mass spectrometry

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ABSTRACT

Developed were the determination methods of Cr^{VI} and Cr^{III} by electrospray ionization mass spectrometry, where $HCrO_4^-$ (m/z 117) and $[Cr^{III}(cydta)]^-$ (m/z 394) were measured, respectively. Moreover, total Cr was also determined by measuring $[Cr^{III}(cydta)]^-$ after reduction of Cr^{VI} with ascorbic acid. Here, cydta denotes *trans*-1,2-diaminocyclohexane-*N,N,N′,N′*-tetraacetic acid. The detection limits were ca. 13 nmol/dm^3 for Cr^{VI} and 56 nmol/dm^3 for Cr^{III} and total Cr. The proposed methods were applied to analyze chromate and trivalent chromium conversion coatings and gave consistent results with those obtained by a diphenylcarbazide spectrophotometric method for Cr^{VI} and ICP-AES for total Cr.

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1. Introduction

Chromium usually exists in the environment as trivalent and hexavalent forms. Although Cr^{III} is considered an essential element for humans and animals, Cr^{VI} is much more toxic than Cr^{III} for both acute and chronic exposure. Thus, recently, amounts of Cr^{VI} in industrially produced samples have been strictly regulated in various fields and countries [1–3]. In particular, Cr^{VI} is one of six materials whose uses are regulated by the RoHS directive of European Union [1]. Because of such circumstances, the analysis for Cr^{VI} has been intensively studied [4–7], on which several reviews have been published recently [8–10]. Although the diphenylcarbazide (DPC) spectrophotometric method has been adopted as a standard method for Cr^{VI} by several public organizations [11–13], an alternative method is still required because of its limitations in terms of the sensitivity and the selectivity.

Recently, we have proposed a new method for the quantitative trace analyses for metallic and nonmetallic ions by electrospray ionization mass spectrometry (ESI-MS), where metal-aminopolycarboxylate (metal-APC) complexes, particularly those of *trans*-1,2-diaminocyclohexane-*N,N,N′,N′*-tetraacetic acid (CyDTA), were measured for metal ion determinations, and the ternary complex of aluminum, fluoride and nitrilotriactic acid (NTA), i.e., $[AlF(nta)]^-$ was measured for fluoride determination after the on-line separation of the complexes from sample matrices with a size exclusion column [14]. Furthermore, the concept of the use of ternary complex formations has been extended to other halides and cyanide determinations [15].

In this study, we newly developed the determination methods for Cr^{VI} and Cr^{III} by electrospray ionization mass spectrometry, where $HCrO_4^-$ (m/z 117) and $[Cr^{III}(cydta)]^-$ (m/z 394) were measured for the determination of Cr^{VI} and Cr^{III}, respectively. Moreover, total Cr was also determined by measuring $[Cr^{III}(cydta)]^-$ after reduction of Cr^{VI} with ascorbic acid. Then, the proposed methods were applied to analyze chromate and trivalent chromium conversion coatings and gave consistent results with those obtained by the DPC spectrophotometric method for Cr^{VI} and ICP-AES for total Cr.

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2. Experimental

2.1. Reagents and chemicals

Potassium dichromate ($K_2Cr_2O_7$) and chromium(III) nitrate nonahydrate ($Cr(NO_3)_3 9H_2O$) (Analytical Grade, Wako Pure Chemicals, Japan) were used as standards for Cr^{VI} and Cr^{III}, respectively. The concentration of Cr^{III} standard solution was checked with Cr^{VI} standard solution by ICP-AES before use. N-(2-hydroxyethyl)ethylenediamine-N,N′,N′-triacetic acid (HEDTA), ethylenediamine-N,N,N′,N′-tetraacetic acid (EDTA), trans-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid (CyDTA), diethylenetriamine-N,N,N′,N″,N″-pentaacetic acid (DTPA) (Dojindo Laboratories, Japan) were used as chelating agents. Diphenylcarbazide (1,5-diphenylcarbonohydrazide) was obtained from Wako Pure Chemicals, Japan. Other reagents of analytical grade were also obtained from Wako Pure Chemicals, Japan. Water was purified by a Millipore Milli-Q system (Millipore Corp., USA). Potassium salt of Co^{III}-edta complex was synthesized according to the reference [16], and was used as an internal standard material.

2.2. Apparatus

The LC-ESI-quadrupole MS system, LCMS-2010 (Shimadzu Co., Japan), was used in flow injection mode. The negative ion mode was used throughout in this study. The instrumental conditions were as follows: electrospray voltage, −3.5 kV; Q-array voltage, −50 V; nebulizer gas (N_2) pressure, 0.1 MPa; the curve desolvation line (CDL) temperature, 300 °C; block heater temperature, 200 °C. No corrosion of the instrument due to Cr^{VI} has been observed and usual maintenance and clean-up of the instrument have been enough so far. Moreover, no increase in background signals due to chromium contaminations have not been observed so far. Sample solutions were introduced with a six-way loop injector whose sample loop volume was 30 mm^3. Pure water was used as a carrier solution (flow rate, 0.2 cm^3 min^{-1}). For all the Cr measurements, the ion intensities of ^{52}Cr (natural abundance, 83.8%), i.e., $H^{52}CrO_4^-$ (m/z 117) and $[^{52}Cr^{III}(cydta)]^-$ (m/z 394), were used. The day-to-day variation of the ion intensities themselves was typically ca. 10–20%, thus, the internal standard, $[Co^{III}$-edta$]^-$ (m/z 347), was used for the determination. The use of the internal standard and the standard addition method has made the influence of such variation minimum [14,15].

2.3. Samples, sample preparation and quantification

Two types of zinc-plated iron plates (2.5 cm × 3.0 cm), on which chromate conversion coating (yellow) and trivalent chromium conversion coating (blue) were performed respectively, were used as actual samples. They were prepared for an inter-laboratory test organized by NMIJ. For extractions of Cr^{III} and Cr^{VI}, two pieces of the plates were put in ca. 50 cm^3 of gently boiling ultrapure water for 30 min according to the protocol of the inter-laboratory test. After cooling, the water samples were made up to 50 cm^3. Here, the sample solution is named "the sample A". An aliquot of the sample A was further diluted five times (the sample B). The analytical results were expressed as the amounts of Cr per surface area of the plate (μg cm^{-2}). The total surface area of the two pieces of the plates was 30 cm^2. The samples A and B were used for the following analyses.

2.4. Determination of Cr^{VI} by ESI-MS

2 cm^3 of the sample B was mixed with the same volume of the solution containing 2 mmol/dm^3 of ammonium acetate, 40 μmol/dm^3 of Co-edta as an internal standard, 0, 2.0, 5.0, or 10.0 μmol/dm^3 of potassium dichromate (pH was adjusted to 5 with a small amount of acetic acid–ammonium acetate buffer). The series of the sample solutions were used for ESI-MS measurements, where the ion intensities of $HCrO_4^-$ (m/z 117) and internal standard $\{[Co^{III}$-edta$]^-$ (m/z 347)$\}$ of the sample solutions were simultaneously monitored with single ion mode (SIM). Each sample solution was measured five times and the quantification was performed by a standard addition method based on the intensity ratio of $HCrO_4^-$ (m/z 117) and $[Co^{III}$-edta$]^-$ (m/z 347).

2.5. Determination of Cr^{III} by ESI-MS

2 cm^3 of the sample A or B was mixed with the same volume of the solution containing 2 mmol/dm^3 of ammonium acetate, 40 μmol/dm^3 of Co-edta as an internal standard, 0, 0.5, 1.0, or 2.0 μmol/dm^3 of chromium(III) nitrate, 400 μmol/dm^3 of CyDTA (pH was adjusted to 5 with a small amount of acetic acid–ammonium acetate buffer). The series of the sample solutions were warmed to 40 °C for 1 h to complete the complex formation, then, were used for ESI-MS measurements, where the ion intensities of $HCrO_4^-$ (m/z 117) and internal standard $\{[Co^{III}$-edta$]^-$ (m/z 347)$\}$ of the sample solutions were simultaneously monitored with SIM. Each sample solution was measured five times and the quantification was performed by a standard addition method based on the intensity ratio of $HCrO_4^-$ (m/z 117) and $[Co^{III}$-edta$]^-$ (m/z 347).

2.6. Determination of total Cr by ESI-MS

2 cm^3 of the sample B was mixed with the same volume of the solution containing 2 mmol/dm^3 of ammonium acetate, 40 μmol/dm^3 of Co-edta as an internal standard, 0, 2.5, 5.0, or 10.0 μmol/dm^3 of potassium dichromate, 400 μmol/dm^3 of CyDTA, and 1 mmol/dm^3 of ascorbic acid (pH was adjusted to 5 with a small amount of acetic acid–ammonium acetate buffer). The series of the sample solutions were warmed to 40 °C for 1 h to complete the complex formation, then, were used for ESI-MS measurements, where the ion intensities of $HCrO_4^-$ (m/z 117) and internal standard $\{[Co^{III}$-edta$]^-$ (m/z 347)$\}$ of the sample solutions were simultaneously monitored with SIM. Each sample solution was measured five times and the quantification was performed by a standard addition method based on the intensity ratio of $HCrO_4^-$ (m/z 117) and $[Co^{III}$-edta$]^-$ (m/z 347).

2.7. Determination of Cr^{VI} and total Cr by a DPC method and ICP-AES

Cr^{VI} was determined using the sample A according to Ref. [13]. Moreover, total Cr was also determined by ICP-AES. Cr in the sample A or B was determined by the calibration curve method, where the analytical line was 267.72 nm.

3. Results and discussion

3.1. Determination of Cr^{VI} by ESI-MS

Fig. 1 shows the ESI mass spectrum of 0.1 mmol/dm^3 of potassium dichromate solution at pH 5. The peaks of $Cr_2O_7^{2-}$ (m/z 108), $HCrO_4^-$ (m/z 117), $HCr_2O_7^-$ (m/z 217) were found on the spectrum. The signal of CrO_4^{2-} (m/z 59) was overlapped by that of acetate (m/z 59) in this study. As shown in the figure, $HCrO_4^-$ ion (m/z 117) gave the highest peak. Then, the effect of solution pH on the spectrum was examined; the highest sensitivity for Cr^{VI} was obtained in the range of pH 4 to 5. Thus, $HCrO_4^-$ ion (m/z 117) was measured at pH 5 for Cr^{VI} quantification. Linear response was obtained at least up to 50 μmol/dm^3 of chromium(VI). Moreover, the limit of detection (LOD, 3σ of the noise level) was ca. 13 nmol/dm^3 (for Cr), which is about one order of magnitude lower than that of the DPC method.
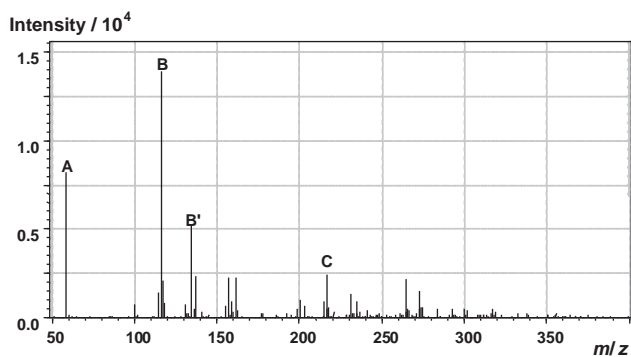
Fig. 1. ESI mass spectrum of 0.1 mmol/dm^3 of potassium dichromate solution (pH 5). The sample solution also contained 1 mmol/dm^3 of ammonium acetate. (A) CH_3COO^- (*m/z* 59) + CrO_4^{2-} (*m/z* 59); (B) $HCrO_4^-$ (*m/z* 117); (B') $(HCrO_4 + H_2O)^-$ (*m/z* 135); (C) $HCr_2O_7^-$ (*m/z* 217).

Table 1
Reduction percentage of Cr^{VI} to Cr^{III} during complex formation reactions.

Chelating reagent	Reduction percentage (%)
HEDTA	2.2
EDTA	15
CyDTA	0.57
DTPA	0.65

The solution containing 50 μmol/dm^3 of Cr^{VI} and 200 μmol/dm^3 of each chelating reagent was warmed to 40 °C for 1 h, then, the corresponding Cr^{III} complex was measured by ESI-MS.

3.2. Determination of Cr^{III} by ESI-MS

According to our previous work [14], we tried to detect Cr^{III} as Cr^{III}-APC complexes and HEDTA, EDTA, CyDTA, DTPA were examined. The solutions containing zero to 50 μmol/dm^3 of Cr^{III} and 200 μmol/dm^3 of respective APCs were prepared and ion intensities of those Cr^{III}-APC complexes, i.e., $[Cr^{III}(hedta)_{-1}]^-$ (*m/z* 326), $[Cr^{III}(edta)]^-$ (*m/z* 340), $[Cr^{III}(cydta)]^-$ (*m/z* 394), and $[Cr^{III}(Hdtpa)]^-$ (*m/z* 441), in ESI-MS were compared with one another. Although it is well known that the formation rate of the Cr^{III} complexes is usually very slow, the reaction condition of 40 °C for 1 h was enough for all cases tested. Moreover, all of Cr^{III}-APC complexes tested gave linear response to Cr^{III} concentration. Among them, EDTA and CyDTA gave the highest sensitivity for Cr^{III}; the ratio of the signal intensities was about 10:10:6:4 for EDTA:CyDTA:DTPA:HEDTA against the same concentration of Cr^{III}. Then, it was tested whether the reduction reaction of Cr^{VI} would occur during the complex formation. The results were summarized in Table 1. The reduction percentage (%) was calculated from the ratio of the ESI-MS intensity of each Cr^{III}-APC complex for the sample containing 50 μmol/dm^3 of Cr^{VI} to that for the sample containing the same concentration of Cr^{III}. As shown in the table, considerable amounts of Cr^{VI} (15%) were reduced to Cr^{III} and form Cr^{III}-edta complex in the case of EDTA, while very small amounts of Cr^{VI} (0.57%) were reduced in the case of CyDTA. The similar results were obtained in the range of pH 5–7 for both cases. Thus, CyDTA was chosen for Cr^{III} determination. Fig. 2 shows the ESI mass spectrum of the solution containing 50 μmol/dm^3 of Cr^{III} and 200 μmol/dm^3 of CyDTA (pH 5). The ion intensity of $[^{52}Cr^{III}(cydta)]^-$ (*m/z* 394) was used for the Cr^{III} determination. The LOD for Cr^{III} was 56 nmol/dm^3. Linear response was obtained at least up to 50 μmol/dm^3 of Cr^{III}.

3.3. Determination of total Cr by ESI-MS

Then, the determination of total Cr was performed by ESI-MS, where all of Cr^{VI} should be reduced to Cr^{III} with the addition of ascorbic acid during the complex formation with CyDTA. The effect of the concentration of ascorbic acid on the reduction efficiency was examined for 50 μmol/dm^3 of Cr^{VI} solution. As a result, the quantitative reduction of Cr^{VI} was obtained with the addition of no less than 500 μmol/dm^3 of ascorbic acid. Moreover, there was no influence of the addition of 500 μmol/dm^3 ascorbic acid with ESI-MS measurements. The ESI-MS intensity of Cr^{III}-cydta obtained from the reduction of 50 μM of Cr^{VI} showed the same intensity as that obtained from 50 μM of Cr^{III}. Thus, the procedure mentioned in *Experimental* was adopted to determine total Cr by ESI-MS. The LOD for total Cr was the same as that for Cr^{III}, i.e., 56 nmol/dm^3.

3.4. Determination of Cr^{III}, Cr^{VI} and total Cr in chromate and trivalent chromium conversion coatings

The methods were applied to determine Cr^{VI}, Cr^{III} and total Cr in chromate and trivalent chromium conversion coatings. Moreover, the DPC method and ICP-AES were also applied to determine Cr^{VI} and total Cr, respectively, as reference methods. The results are summarized in Table 2. The samples #1 and #2 were "chromate conversion coating" and "trivalent chromium conversion coating", respectively. For the sample #1, the analytical values of the present methods were in good agreement with those of the reference methods. Fig. 3 shows the standard addtion method for the detrmination of Cr^{VI} by ESI-MS (without CyDTA) for the sample #1. Relative standard deviations of the measurement points were 1.7–3.7% and the overall error for the analytical value was 7.0%, i.e., 2.45 ± 0.17 μmol/dm^3: this value (the molar concentration of Cr^{VI} in the sample B) was converted to the value (2.13 ± 0.15 μg/cm^2) in Table 2, i.e., Cr^{VI} amounts per the surface area. The Cr^{VI}
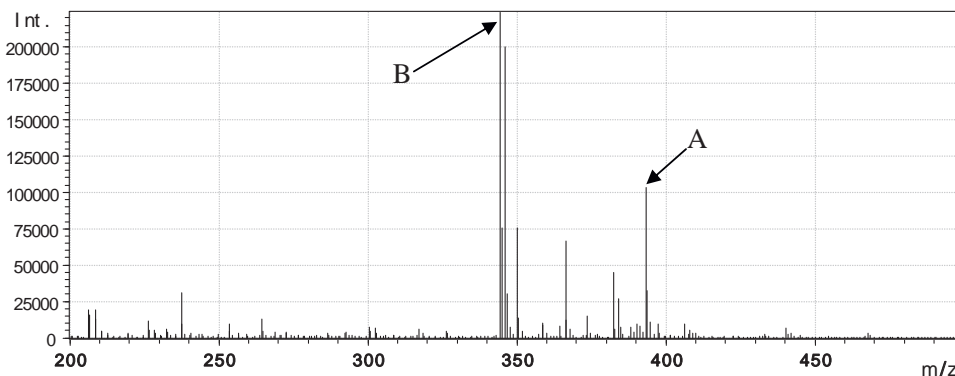

Fig. 2. ESI mass spectrum of the solution containing 50 μmol/dm^3 of Cr^{III} and 200 μmol/dm^3 of CyDTA (pH 5). The sample solution also contained 1 mmol/dm^3 of ammonium acetate. (A) $[Cr^{III}(cydta)]^-$ (*m/z* 394) and (B) H_3cydta.

Table 2
Determination of Cr^{III}, Cr^{VI} and total Cr in chromate conversion coatings by electrospray ionization mass spectrometry.

Analyte	Method	Amounts ($\mu g/cm^2$)[a]	
		Sample #1[b]	Sample #2[c]
Cr^{III}	ESI-MS {$[Cr^{III}(cydta)]^-$ (m/z 394)}[d]	0.012 ± 0.003	–
Cr^{VI}	ESI-MS [$HCrO_4^-$ (m/z 117) without CyDTA]	2.13 ± 0.15	<0.001
	ESI-MS [$HCrO_4^-$ (m/z 117) with CyDTA][d]	1.88 ± 0.14	–
	DPC spectrophotometry	2.03	<0.005
Total Cr	ESI-MS {$[Cr^{III}(cydta)]^-$ (m/z 394)}[e]	1.85 ± 0.04	<0.005
	ICP-AES	1.96 ± 0.03	<0.011

[a] The analytical values by ESI-MS were obtained by a standard addition method based on the relative intensities to those of the internal standard, $[Co^{III}\text{-edta}]^-$ (m/z 347), where each measurement point was the average of five times measurements. In DPC spectrophotometry and ICP-AES, a calibration curve method was used. See Section 2 for detail.

[b] Hexavalent chromate coating (surface area, $30\,cm^2$, see Section 2 for detail).

[c] Trivalent chromium coating (surface area, $30\,cm^2$, see Section 2 for detail).

[d] Two cm^3 of the sample A was mixed with the same volume of the solution containing 2 mmol/dm^3 of ammonium acetate, 40 μmol/dm^3 of Co-edta as an internal standard, 0, 2.0, 5.0, or 10.0 μmol/dm^3 of potassium dichromate, 400 μmol/dm^3 of CyDTA (pH was adjusted to 5 with acetic acid–ammonium acetate buffer). The series of the sample solutions were warmed to 40 °C for 1 h to complete the complex formation, then, were used for ESI-MS measurements, where the ion intensities of $HCrO_4^-$ (m/z 117) and internal standard {$[Co^{III}\text{-edta}]^-$ (m/z 347)} of the sample solutions were simultaneously monitored with SIM.

[e] Cr^{VI} was reduced to Cr^{III} with the addition of 500 μmol/dm^3 of ascorbic acid.

determination with the sample containing 200 μmol/dm^3 of CyDTA also gave consistent result ($1.88 \pm 0.14\,\mu g/cm^2$) with those of other methods, although the ion intensity of $HCrO_4^-$ (m/z 117) itself decreased to ca. 70% of that without CyDTA. The use of the internal standard and the standard addition method made its influence minimum. These results may prove the repeatability and the stability of the present methods. Although a very small amount of Cr^{III} ($0.012\,\mu g/cm^2$) was also found in the sample #1, it might have come from the reduced Cr^{VI} during the complex formation. The value correspond to 0.6% of that of Cr^{VI} ($2.13\,\mu g/cm^2$), which is the same as the value of Table 1. Thus, we should conclude that no substantial Cr^{III} was contained in the sample #1. As for the sample #2, no Cr was found in the extraction solution. In our preliminary study, another trivalent chromium conversion coating plate was analyzed, and considerable amounts of Cr were detected with our method (Cr^{III} and total Cr) and ICP-AES (total Cr), i.e., 9.95 $\mu g/cm^2$ for Cr^{III} and 10.1 $\mu g/cm^2$ for total Cr by ESI-MS and 10.4 $\mu g/cm^2$ for total Cr by ICP-AES. Although the reason for the difference is not clear, the usefulness of the present method for Cr^{III} and total Cr may be proved by the results for the sample #1 and the preliminary result.

Fig. 3. Standard addition method for the determination of Cr^{VI} extracted from chromate conversion coating plates (sample #1). Y-axis is the intensity ratio of $HCrO_4^-$ (m/z 117) and the internal standard (IS), $[Co^{III}\text{-edta}]^-$ (m/z 347). The number in ( ) is relative standard deviation (%) of the measurements. See Section 2.4 of *Experimental* for further experimental conditions.

4. Conclusions

We newly developed the determination method of Cr^{III}, Cr^{VI} and total Cr by ESI-MS. It is sensitive, rapid and easy to perform. Thus, the method can provide a useful alternative method for the Cr analysis of chromate and trivalent chromium conversion coatings. Moreover, this method has potentiality to provide a rapid simultaneous determination method of nmol/dm^3 or fewer levels of Cr^{III} and Cr^{VI} in natural samples. Presently, however, we may need further study to apply this method to analyze other environmental samples such as soils and natural waters, considering their matrix effects, thus such study is now being performed in our laboratory.

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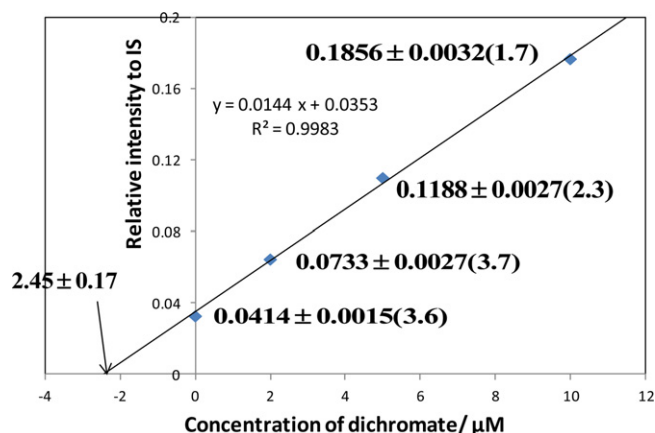[16] F.P. Dwyer, E.C. Gyarfas, D.P. Meller, J. Phys. Chem. 59 (1955) 296–297.