Simultaneous Determinations of Cr(VI) and Cr(III) by Ion-Exclusion/Cation-Exchange Chromatography with an Unmodified Silica-Gel Column

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In order to characterize the ion-exclusion and cation-exchange properties of an unmodified silica-gel column, the retention behaviors of Cr(VI) and Cr(III) ions were investigated using a Develosil 30-5 (150 × 4.6 mm i.d.) in the acidic region. Cr(VI) was separated from other anions by an ion-exclusion and ion-adsorption mechanism, and Cr(III) was separated from other cations with a cation-exchange mechanism. When using 2.0 mM oxalic acid (pH 2.6) as an eluent, a good separation of Cr(VI) and Cr(III) was obtained using conductimetric detection in 12 min. The method was successfully applied to the simultaneous determinations of Cr(VI) and Cr(III) added into tap-water and river-water samples.

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Chromium is a naturally occurring element found in rocks, animals, plants, soil, and volcanic dust and gases. Also, Cr(VI) and Cr(III) enter the environment as a result of effluent discharge from steel industries, electroplating, tanning industries, oxidative dyeing, and chemical industries. Cr(III) compounds are considered to be essential trace elements in the human body, playing roles in the metabolism of glucose and certain lipids, mainly cholesterol.^{1,2} On the other hand, owing to the high oxidation potential and capability of the penetrating power of biological membranes, Cr(VI) compounds are toxic to humans and have a potential to be carcinogens.³

Therefore, the concentrations of Cr(VI) in drinking water and industrial waste water and materials in electrical and electronic equipment are restricted by various measures. A maximum permissible concentration for Cr(VI) in environment water is prescribed legally to be 0.05 mg L⁻¹ by Ministry of the Environment in Japan; that in electrical and electronic equipment is prescribed legally to be 1000 mg kg⁻¹ by RoHS (Restriction of Hazardous Substances) and WEEE (Waste Electrical and Electric Equipment) in Europe.

For the determination of Cr(VI) and Cr(III), ion chromatography (IC), spectrophotometry,⁴ atomic absorption spectrometry (AAS),⁵⁻⁷ inductively coupled plasma atomic emission spectrometry (ICP-AES)⁸⁻¹⁰ and inductively coupled plasma mass spectrometry (ICP-MS)¹¹⁻¹³ are commonly used. On-line selective and simultaneous determinations for both species have been successfully achieved by chromatographic techniques coupled with AAS, ICP-AES and ICP-MS.

For the separation of Cr(VI) and Cr(III), mainly solvent extraction,¹⁴ coprecipitation,¹⁵ ion-exchange separation,¹⁶ adsorption on iminodiacetic chelate resins^{8,9} and on active

alumina^{5,10} and electrodeposition¹⁷ are mainly used. Ion chromatography and high-performance liquid chromatography of Cr(VI) and Cr(III) are included in the following techniques: (1) separation of Cr(VI) by an anion-exchange resin and separation of Cr(III) by cation-exchange resins; (2) complexation of Cr(III) with EDTA is used, and this reaction causes formation of the [Cr(III)-EDTA]⁻ anion species. Both the Cr(III)-EDTA complex and Cr(VI) (Cr₂O₇²⁻ anion species) are separated by reversed-phase ion-pair chromatography using an ion-pair reagent, such as tetrabutylammonium ion. Then, both species are detected by ICP-MS.¹⁸⁻²⁰ In these separations, the Cr(III)-EDTA species is eluted earlier, followed by the Cr(VI) species.

Ion chromatography is a very effective method for determining both anions and cations in many types of natural water. Especially, simultaneous ion-exclusion/cation-exchange chromatography (IEC/CEC) using an acidic eluent on a single separation column has been recognized to be an effective method to determine anions and cations simultaneously.²¹⁻²⁵

Silica-gel is widely used as a packing material for high-performance liquid chromatography. Silica gel has a silanol group, which is known to behave as a weak acid with a pK_a of 7.1.²⁶ Smith and Pietrzyk,²⁷ Brown and Pietrzyk,²⁸ Iwachido *et al.*,²⁹⁻³¹ and Ohta³² have used silica gel as a cation-exchange stationary phase for IC with conductimetric detection for the separation of common mono- and divalent cations, such as alkali metal, ammonium and alkaline earth metal ions.

In the present study, the simultaneous determinations of Cr(VI) and Cr(III) using an unmodified silica-gel column with a conductimetric detection was established. In this separation, the Cr(VI) species was separated by IEC, and the Cr(III) ion was eluted by CEC from an unmodified silica-gel column with oxalic acid used as the eluent. In this separation, Cr(VI) eluted earlier from the column, and Cr(III) followed. For this separation mechanism there is no report as far as we know.

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Fig. 1 Effect of the concentration of oxalic acid on the retention volumes of Cr(VI). Conditions: eluent, 0.5–3.0 mM oxalic acid; flow rate, 1 mL min⁻¹; column, Develosil 30-5 (150 × 4.6 mm i.d.); column temperature, 30°C; injection volume, 20 µL; sample concentration, 0.5 mM Cr(VI) (□), 1.0 mM Cr(VI) (◆).

Experimental

Instrumentation

A porous spherical silica-gel column, Develosil 30-5 (150 × 4.6 mm i.d., 5 µm particle size), was purchased from Nomura Chemicals (Seto, Japan). The ion chromatograph consisted of a Jasco PU-980 pump (Tokyo, Japan), a Shimadzu CTO-10A vp column oven, a Shimadzu CDD-6A conductimetric detector and a Rheodyne (Cotati, CA) 77251 injector with a 20-µL sample loop. A Jasco PU-980 pump was operated at a flow rate of 1 mL min⁻¹, a Shimadzu CTO-10A vp column oven was operated at 30°C, and a Shimadzu Class-VP (Ver. 5.03) chromatographic data-processor was used.

Reagent

All reagents were of analytical reagent-grade. Distilled, deionized water was used for the preparation of standard solutions and eluents. The eluent for ion chromatographic separations was analytical-reagent grade oxalic acid (Wako Pure Chemical Industries, Osaka, Japan) diluted with high-purity water from a Milli-Q/SP system (Nihon Millipore, Tokyo, Japan). The 1000 ppm of Cr(VI) standard was purchased from Wako Pure Chemicals, and the 1000 ppm of Cr(III) standard was purchased from Merck (Darmstadt, Germany).

Results and Discussion

Separation of Cr(VI) by ion-exclusion

The elution behavior of Cr(VI) ion was investigated using oxalic acid as an eluent. In the acidic medium Cr(VI) ion exists in the form of Cr₂O₇²⁻ anion. Therefore, using oxalic acid as the eluent, Cr(VI) ion was ion-excluded quickly from the silica-gel column. Using 0.5 mM (pH 3.7)–3.0 mM (pH 2.5) oxalic acid as the eluent, the elution volume of Cr(VI) was investigated using 0.5 and 1.0 mM Cr(VI) standard solutions. The retention volume of Cr(VI) gradually increased with increasing the concentration of oxalic acid, as shown in Fig. 1. From Fig. 1, it is considered that Cr(VI) anion eluted by means of both the ion-exclusion effect and the ion-adsorption effect.

Separation of Cr(III) by cation-exchange

Cation-exchange on silica-gel is normally performed using a weak acidic or neutral eluents and with conductimetric detection. Ohta *et al.*³² reported that by using 0.25–5 mM nitric acid as

Fig. 2 Ion chromatogram of mono-, di- and trivalent ions. 1, Na⁺; 2, NH₄⁺; 3, K⁺; 4, Mg²⁺; 5, Ca²⁺ 0.25 mM; 6, Cr(III) 0.50 mM. Conditions: eluent, 1.5 mM oxalic acid; column, Develosil 30-5 (150 × 4.6 mm i.d.); injection volume, 20 µL; 1 V = 100 µS cm⁻¹.

Fig. 3 Effect of the concentration of oxalic acid on the retention volumes of Cr(III). Conditions: eluent, 1.0–3.0 mM oxalic acid; flow rate, 1 mL min⁻¹; column, Develosil 30-5 (150 × 4.6 mm i.d.); column temperature, 30°C; injection volume, 20 µL; sample concentration, 0.5 mM.

the eluent, five common mono- and divalent cations (Na⁺, NH₄⁺, K⁺, Mg²⁺ and Ca²⁺) were consequently eluted on a silica-gel column, Develosil 30-5, based on cation-exchange reactions. From these data, it is expected that the mono-, di- and trivalent cations will be separated simultaneously on the silica stationary phase using a weak acid as the eluent based on the difference in the affinity of mono-, di-, and trivalent cations for the ionized silanol cation-exchange sites. Using oxalic acid (pK_{a1} = 1.27, pK_{a2} = 4.27) as the eluent, elutions of mono- (Na⁺, NH₄⁺ and K⁺), di- (Mg²⁺ and Ca²⁺) and trivalent (Cr³⁺) cations were investigated. As shown in Fig. 2, the use of 1.5 mM oxalic acid as the eluent gave good separations of the mono-, di- and trivalent cations on a Develosil 30-5 column.

Using 1.0–3.0 mM oxalic acid as the eluent, the retention volumes of Cr(III) was investigated. As shown in Fig. 3, the retention volumes of Cr(III) decreased with an increase of the oxalic acid concentration. Using 1.8–3.0 mM oxalic acid as the eluent, Cr(III) eluted from the column within 12 min and 1.8–2.5 mM concentrations of oxalic acid as the eluent were considered to be appropriate for the separation of monovalent, divalent and Cr(III) cations.

From chromatographic separations of Cr(VI) (Fig. 1) and Cr(III) (Fig. 3), the optimum concentration of oxalic acid as the eluent was judged to be 2.0 mM for simultaneous separations of Cr(VI) and Cr(III) ions.

Analytical performance parameters

A typical chromatogram of Cr(VI) and Cr(III) is shown in Fig. 4. The peak of Cr(VI) appeared to be positive based on the

Fig. 4 Chromatogram of Cr(VI) and Cr(III) ions using oxalic acid as the eluent. Conditions: eluent, 2.0 mM oxalic acid; flow rate, 1 mL min⁻¹; column, Develosil 30-5 (150 × 4.6 mm i.d.); column temperature, 30°C; injection volume, 20 µL; sample concentration, 0.5 mM each; 1, strong acid anions.

Table 1 Analytical performance of Cr(VI) and Cr(III) by IEC/CEC

Metal	Range/mM	Linearity	DL/µM	Precision, %
Cr(III)	0.1 – 0.5	0.9976	3.56	1.19
Cr(VI)	0.1 – 1.0	0.9938	1.40	0.47

DL, Detection limit; IEC, ion-exclusion chromatography; CEC, cation-exchange chromatography. Precision: the relative standard deviation of peak areas in the repeated chromatographic runs ($n = 4$) of a 0.5 mM standard solution of Cr(III) or a 0.5 mM standard solution of Cr(VI).

addition of signals of $\text{Cr}_2\text{O}_7^{2-}$ in the sample to the background signals of oxalic acid, while the peak of Cr(III) appeared to be negative based on the difference in the equivalent conductance between the Cr^{3+} ion in the sample and the H^+ ion generated from the dissociation of oxalic acid, according to an indirect conductivity detection of anions and cations.^{21–25} Calibration graphs were obtained by plotting the peak area against the concentration of the anion and cations. Linear calibration graphs were obtained in at least the concentration ranges 0.1 – 1.0 mM for Cr(VI) and 0.1 – 0.5 mM for Cr(III) ions. The detection limits calculated at $S/N = 3$ were 1.40 µM (72.9 ppb) for Cr(VI) and 3.56 µM (185.0 ppb) for Cr(III), respectively. The relative standard deviation (RSD) of the peak areas in the repeated chromatographic runs ($n = 4$) using a 0.5 mM Cr(VI) standard solution or a 0.5 mM of Cr(III) standard solution, under the optimum chromatographic conditions was less than 1.1% ($n = 4$). The analytical performance of the method is indicated in Table 1. One sample could be analyzed within 12 min.

Interference effects were tested for the common anions and cations. Table 2 gives the retention volumes of various anions and cations under the optimum chromatographic conditions. Strong acids anions, such as Cl^- , NO_3^- and SO_4^{2-} ions, were eluted closely at the completed ion-exclusion volume of the column, and Cr(VI) was eluted at 2.56 min after the eluent-dip peak due to both ion-exclusion and ion-adsorption effects. However, Cr(III) was eluted at 8.3 min after the elution of common mono- and divalent cations by the cation-exchange effect. Heavy metals were eluted as appropriate in wide retention volumes based on the stability constants with oxalic acid. From Table 2, the retention volume of Cu^{2+} was 1.41 mL which is very small. The stability constant of Cu^{2+} with oxalic acid was reported to be $10^{6.3}$.³³ Therefore, it is considered that the Cu^{2+} ion complexed with oxalic acid and formed a large

Table 2 Retention volume (V_R) of various ions under the optimum chromatographic conditions^a

Anion	V_R/mL	Cation	V_R/mL	Cation	V_R/mL
Cl^-	1.29	Li^+	3.13	Co^{2+}	2.74
NO_3^-	1.31	Na^+	3.56	Cd^{2+}	4.39
SO_4^{2-}	1.42	NH_4^+	3.96	Mn^{2+}	4.47
$\text{Cr}_2\text{O}_7^{2-}$	2.56	K^+	4.56	Cu^{2+}	1.41
		Mg^{2+}	4.85	Ni^{2+}	2.25
		Ca^{2+}	5.17	Pb^{2+}	2.19
				Zn^{2+}	2.56
				Cr^{3+}	8.28
				Fe^{3+}	N.D.

a. Eluent, 2.0 mM oxalic acid (pH 2.6); flow rate, 1 mL min⁻¹; Cr(VI) and Cr(III), 0.3 mM; N.D., not detected.

Fig. 5 Chromatograms of tap-water and river-water samples. (a) Tap-water added 0.3 mM Cr(III) and 0.3 mM Cr(VI) and (b) river-water added 0.3 mM Cr(III) and 0.3 mM Cr(VI). Conditions: eluent, 2.0 mM oxalic acid; flow rate, 1 mL min⁻¹; column, Develosil 30-5 (150 × 4.6 mm i.d.); column temperature, 30°C; injection volume, 20 µL. 1, Strong acid anions; 2, Na^+ ; 3, K^+ ; 4, Ca^{2+} .

anionic species; as a result, Cu^{2+} ion was ion-excluded from the silica-gel. Also, since the retention volume of Zn^{2+} was close to that of Cr(VI), the content of Zn^{2+} is considered to affect on the determination of Cr(VI).

Application to the analysis of environmental water samples

The proposed IEC/CEC method was applied to the simultaneous determinations of Cr(III) and Cr(VI) ions in two environmental samples. Samples were collected in the city of Higashi-hiroshima, in west Japan, and were analyzed after filtration through a 0.22-µm membrane filter.

Figure 5 shows typical chromatograms for Cr(VI) and Cr(III) ions added to a tap-water sample and a river-water sample. The resolution between analyte cations in these chromatograms was quite satisfactory. Table 3 lists the analytical results and recoveries for the Cr(VI) and Cr(III) ions added to the sample

Table 3 Recovery test of Cr(VI) and Cr(III) added into tap-water and river-water samples ($N = 2$)

	Added		Found		Recovery	
	Cr(VI)/mM	Cr(III)/mM	Cr(VI)/mM	Cr(III)/mM	Cr(VI), %	Cr(III), %
Tap-water	0.300	0	0.305 ± 0.003	0	101.6	0
	0	0.300	0	0.315 ± 0.012	0	104.8
	0.300	0.300	0.285 ± 0.017	0.303 ± 0.002	95.0	100.9
River-water	0.300	0	0.312 ± 0.005	0	104.1	0
	0	0.300	0	0.300 ± 0.001	0	100.1
	0.300	0.300	0.297 ± 0.001	0.293 ± 0.003	99.0	97.6

solutions. The recoveries of Cr(VI) and Cr(III) ions added to the sample solutions were over 95.0%. From these results, it was concluded that the proposed IEC/CEC method offered a simple and convenient IC means for the simultaneous determinations of Cr(VI) and Cr(III) ions using a conductimetric detector.

Conclusion

In this work, the cation-exchange properties of unmodified silica (Develosil 30-5) in the acidic region were investigated, and an IEC/CEC method with a conductimetric detector has been established for the simultaneous determinations of Cr(VI) and Cr(III) ions using an oxalic acid eluent. The proposed method could be successfully applied to the simultaneous determinations of Cr(VI) and Cr(III) ions in environmental samples. Further, the combinations of this IC method with ICP-AES and ICP-MS will obtain further higher sensitivities for the determinations of Cr(VI) and Cr(III), and will expand applications of this method to various environmental samples.

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