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Rapid and decisive determination of Cr^{6+} using electrospray ionization mass spectrometry

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Abstract

Cr^{6+} complexed with diethyldithiocarbamate (DDC) was extracted into 0.1 volume of octanol in the presence of citric acid. The extracted compound was determined to be $CrOH(DDC)_3^+$ by electrospray ionization mass spectrometry. The peak at *m/z* 513 derived from ^{52}Cr was so high that only 5 pg of Cr^{6+} could be determined in 10 min.

Key words: Chromium; Electrospray ionization; Mass spectrometry; Diethyldithiocarbamate

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1. Introduction

Cr^{6+} is readily transported across cell membranes and is known to be more toxic than Cr^{3+} in cell systems. In this respect, speciation of Cr^{6+} and Cr^{3+} in biological fluids and waste waters is required. The chelate 1,5-diphenylcarbazide reacts with only Cr^{6+} resulting in a red-violet complex [1,2]. Using this reaction, a flow injection analysis was adopted to determine Cr^{6+} and Cr^{3+} separately [3]. Unfortunately, Fe^{3+}, Cu^{2+}, Ni^{2+}, V^{4+}, Pb^{2+}, Cr^{3+}, Sn^{2+}, Mo^{6+}, Hg^{2+} and Cl^- interfered with the determination [3].

Mass spectrometry (MS) is a powerful technique for the identification of compounds. Gas chromatography (GC)-MS was applied for the determination of Cr^{3+} [4]. In GC method, however, Cr^{3+} should become a volatile compound after derivatization reaction lasting for 120 min at 110 ℃ before GC separation that took another 30 min [4]. Recently electrospray ionization (ESI) MS began to be applied for the analysis of weakly charged species in liquid. We have found that Cr^{6+} reacts immediately with diethyldithiocarbamate (DDC, $(C_2H_5)_2NCS\overset{-}{S}$), and is extracted into octanol in the presence of some acids showing a characteristic spectrum corresponding to $CrOH(DDC)_3^+$ in ESI-MS.

2. Experimental

Atomic absorption standard solutions (AASS) of CrO_4^{2-} and other metals, and other reagents of analytical grade were obtained from Wako Pure Chemicals, Japan, and $K_2Cr_2O_7$ of analytical grade, from Sigma, USA. The standard solutions of Cr^{6+} were prepared daily by dilution in 0.15 M NaCl from the AASS whose quality was certified for 1 year. K_2CrO_4 from Wako and $K_2Cr_2O_7$ from Sigma were used for reference materials. Cr^{6+} is assayed as follows. To 50 µl of sample or CrO_4^{2-} standard solution of 10^{-8} to 10^{-5} M, 5 µl of 1 M DDC is added and mixed for 30 sec by a vortex mixer. The pH of the sample solution should be adjusted around 5 - 9 before the addition of DDC. Five µl of 2 M citric acid is added and mixed for 10 sec. Then, 5 µl of octanol is added and mixed for 10 sec, and centrifuged for 30 sec. ESI-MS was performed by using a TSQ 7000 LC-quardrupole mass spectrometer (Thermo Quest, Japan) in the positive ion mode. One µL of octanol layer was injected manually in the direct mode. The characteristic spectrum appeared 30 sec after sample injection, and sample could be injected every 30 sec. Methanol was flowed as a mobile phase at 200 μ l /min. The capillary voltage was set to be 4.5 kV and the desolvation temperature, 290 ℃. Data were collected in the range of *m/z* 100 – 1000 and the quantitative analysis was conducted using molecular ion at *m/z* 513 in the selected ion monitoring (SIM) mode.

3. Results and discussion

Figures 1 shows an ESI-MS of 1 μl of octanol layer containing 2.5 ng Cr^{6+} measured at the desolvation temperature of 290 ℃, the most suitable temperature. Figs. (a) and (b) indicate the MS at *m/z* 100 – 1000 and 510 – 517, respectively. The highest peak at *m/z* 513 corresponds to $^{52}Cr\ ^{16}O^{1}H\ (^{12}C_5{}^{1}H_{10}{}^{14}N^{32}S_2)_3$ and other peaks, to the mixtures of isotopes of Cr and S with the natural abundances as ^{50}Cr(4%), ^{52}Cr(84%), ^{53}Cr(10%), ^{54}Cr(2%), ^{32}S(95%), ^{33}S(1%) and ^{34}S(4%). Without citric acid, Cr^{6+} complexed with DDC did not show its peaks in positive ion mode. Evidently, a ternary complex is formed among Cr^{6+}, DDC and citric acid [5]. The chromium incorporated into the complex is not Cr^{3+} but Cr^{6+}or Cr^{4+}, as revealed by our electron spin resonance study. The composition of the ternary complex, however, was not determined at present. Cr^{6+} complexed with DDC did not show characteristic peaks measured in negative ion mode either with or without citric acid. Cr^{3+} complexed with DDC did not show its characteristic peaks under the same treatment as that of Cr^{6+}. To quantitate Cr^{3+}, it should be oxidized to Cr^{6+} as reported before [3].

Using octanol as an extractor, citric acid showed the maximum production at 0.1 – 0.4 M, and the production rate decreased to be about 80, 90 and 50 % of the maximum at 0.05, 0.6 and 1.0 M, respectively. The efficiency of 0.2 M citric acid was the highest among those of several acids. That is, those of 0.2 M oxalic acid, ascorbic acid, HCl, HNO_3 and 0.1 M H_2SO_4 were 90, 30, 20, 15, and 15 %, respectively, of that of 0.2 M citric acid. The observed amounts of $CrOH(DDC)_3^+$ in octanol extract after 1 day and 14 days were 100 and 60 % of the starting amount, respectively, at room temperature under room light. The efficiencies of several alcohols such as hexanol, cycohexanol, isoamyl alcohol, pentanol, butanol, with 0.2 M citric acid were 100, 95, 90, 80 and 40 %, respectively, by taking the efficiency of octanol as 100%. Octanol was chosen for the extraction since it not only gave the highest signal but also eliminated water-soluble compounds in biological fluids more efficiently than hexanol.

Figure 2 shows the quantitation of $CrOH(DDC)_3^+$ in SIM mode at *m/z* 513. The detection limit was 5 pg by considering S/N = 3 at the peak. With the limit of quantitation S/N = 10, i.e., 15 pg, the integrated areas of mass chromatogram were proportional to the amounts of Cr up to 500 pg. The present method was compared with the colorimetric method [1,2]. The concentration of Cr^{6+} determined from the absorption at 540 nm (x) and the peak area in SIM at *m/z* 513 (y) were well satisfied with the equation $y = 6.72\ x + 0.10$ with a correlation coefficient of 0.99. The absolute detection limits of Cr in the colorimetry [1,2], the flow injection analysis [3], an inductively coupled plasma (ICP) MS, the GC-MS [4] and the present method were about 150 ng, 250 pg, 1 ng, 1 pg and 5 pg, respectively. ICP-MS that cannot distinguish between Cr^{6+} and Cr^{3+}, has a problem of overlapping of isobars such as ^{50}Ti, ^{50}V and ^{54}Fe with ^{50}Cr and ^{54}Cr. In GC-MS, the compound of $^{54}Fe^{3+}$ coincided with that of $^{54}Cr^{3+}$ [4]. In the present ESI-MS, the valence states as well as the polarity of DDC compounds of transition metals in octanol were reflected in their spectra. Transition metals such as Ti^{4+}, V^{5+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Zr^{4+}, Mo^{6+}, Cd^{2+}, W^{6+} and Pb^{2+} at 10^{-4} M, i.e., about 10 ng each, did not show peaks at *m/z* 513±15 corresponding to their compounds under the same condition as that of Cr^{6+}.

Recoveries were tested using plasma and urine. Since precipitates were formed in plasma, the amount of octanol was set to twice that for standard solution or urine. The total recoveries from plasma spiked at 10^{-7} M and 10^{-6} M Cr^{6+} were 93±5 % and

95±5 % for six determinations, respectively, and were the same as those in urine.

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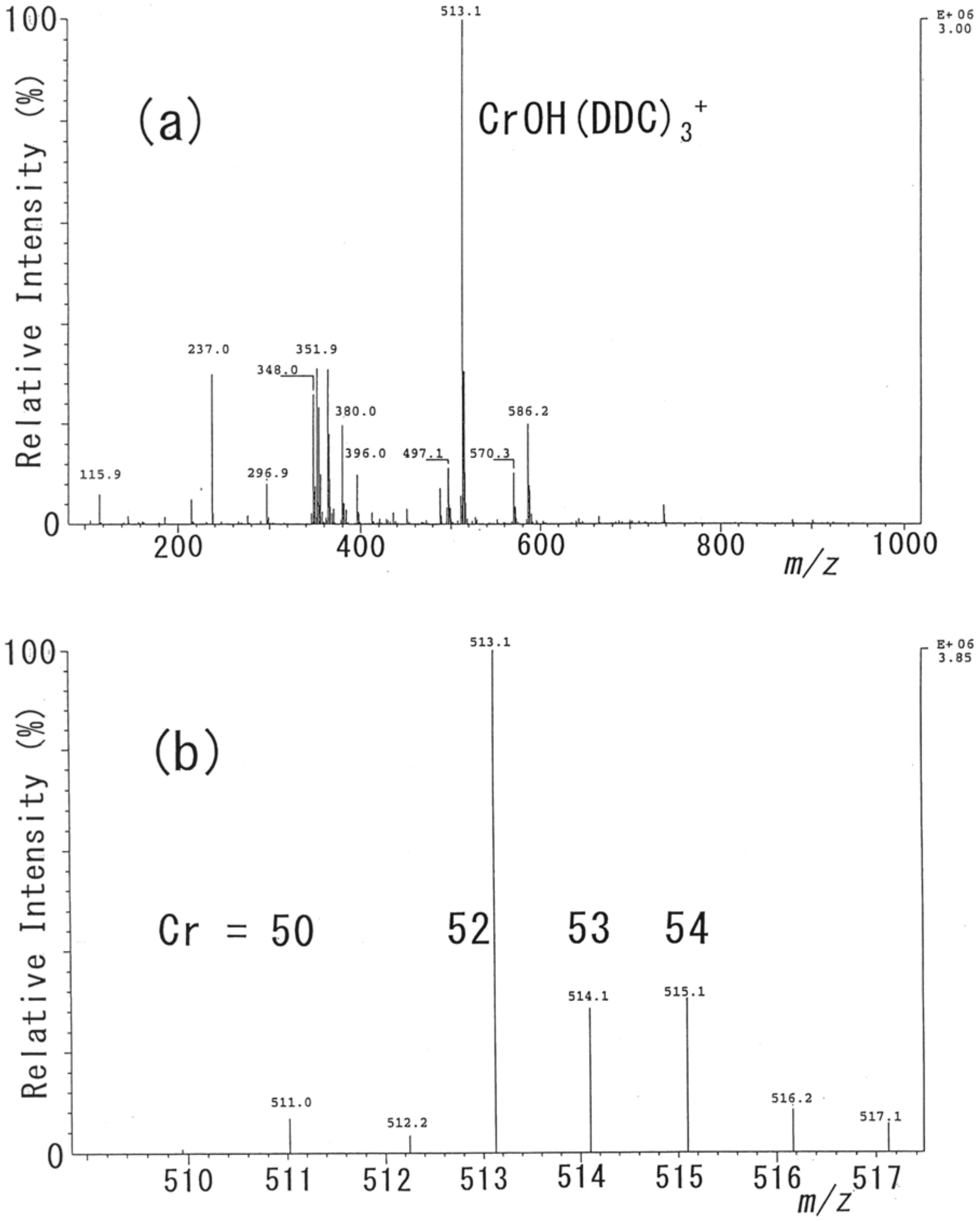
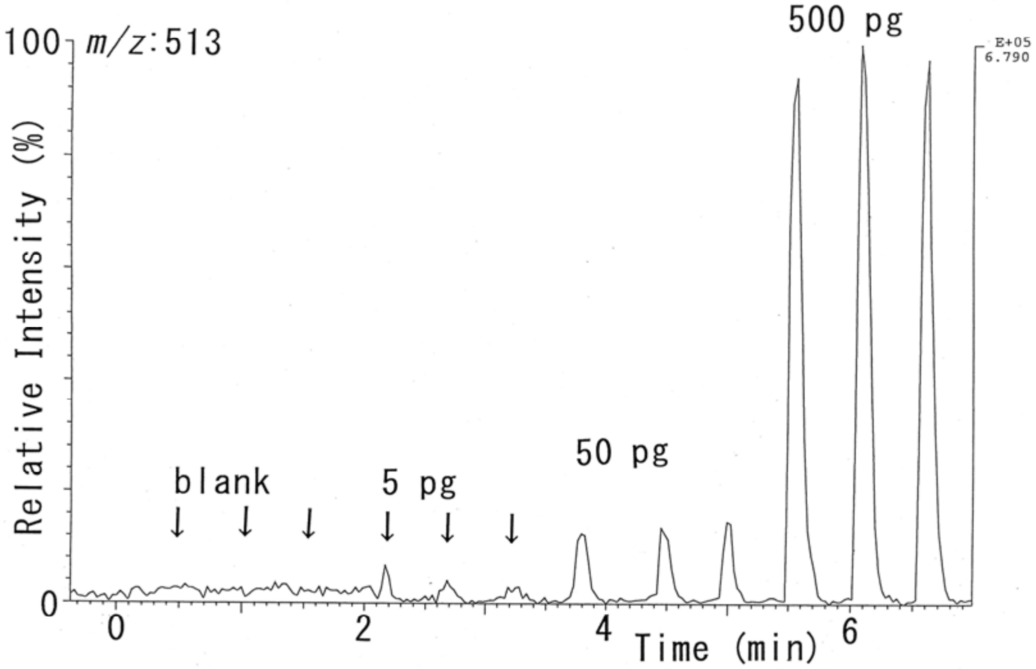
Figure captions

Figure 1. Mass spectra of 1 μl of octanol containing 2.5 ng Cr^{6+}. Figs. (a) and (b) were recorded from *m/z* 100 – 1000 and 510 – 517, respectively.

Figure 2. Chromatogram of $CrOH(DDC)_3^+$ in SIM mode at *m/z* 513. Aliquots of 0, 5, 50 and 500 pg were injected three times for each amount.


(a)
CrOH(DDC)3+
Relative Intensity (%)
100
0
E+ 06
3.00
513.1
237.0
351.9
348.0
380.0
586.2
396.0
497.1
570.3
115.9
296.9
200
400
600
800
1000
m/z
(b)
Relative Intensity (%)
100
0
E+ 06
3.85
513.1
Cr = 50
52
53
54
514.1
515.1
511.0
512.2
516.2
517.1
510
511
512
513
514
515
516
517
m/z


100
m/z:513
500 pg
E+05
6.790
Relative Intensity (%)
blank
5 pg
50 pg
0
0
2
4
6
Time (min)