Speciation of Vanadium(IV) and Vanadium(V) Using Ion-exchange Chromatography and ICP-AES

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Abstract: A speciation method for vanadium(IV) and vanadium(V) is presented that uses a combination of HPLC and ICP-AES. In this method, 1 mM HNO₃ solution and 100 mM HNO₃ solution were applied in sequence as eluent. A vanadium(IV) and vanadium(V) mixture was injected into a HPLC anion-exchange column; and vanadium(IV) cation was then eluted by 1 mM HNO₃, while vanadium(V) oxoacid anion was trapped on the column. After this separation, vanadium(V) was eluted as a cation from the column by 100 mM HNO₃. Vanadium was detected by ICP-AES. In this separation, about 15% of vanadium(V) interfered with vanadium(IV), and trace vanadium(IV) interfered with vanadium(V). This interference could be estimated by simple calculation based on standard observations, and the speciation of vanadium(IV) and vanadium(V) was performed. The lower determination limit was 1 μg/mL, which is insufficient to speciate vanadium sampled by conventional sampling methods in a working environment. However, impurity of the other valent vanadium species in a vanadium(V) reagent can be determined by the present method, which should be valuable in precisely assessing the toxicities of vanadium species.

Keywords: Vanadium — Vanadium pentoxide — Speciation — Ion-exchange chromatography — HPLC — ICP-AES

INTRODUCTION

Vanadium is contained in petroleum and coal, and the combustion of these substances causes vanadium compounds to be released into the surrounding environment. Vanadium alloys are used in various capacities in the machine industry. Vanadium compounds are also used as catalysts in the chemical industry. Chimney sweepers and boiler cleaners may come into contact with vanadium compounds contained in petroleum or coal, and some cases of vanadium toxicosis have been reported[1, 2]. Cases of vanadium toxicosis involving workers engaged in the production of vanadium...
Vanadium pentoxide have also been reported as have cases involving workers engaged in maintenance of vanadium catalysts. Vanadium pentoxide is so toxic as to be designated in Japan as a “Specified chemical substance” under the Enforcement Order of Industrial Safety and Health Law. Employers are obliged under law to control workplace concentration of vanadium pentoxide in order to protect the health of workers. The Japanese administrative level for vanadium pentoxide is 0.03 mg/m³ as vanadium.

Vanadium exhibits various valences, such as vanadium(II), vanadium(III), vanadium(IV) and vanadium(V). Vanadium(II) is unstable in the environment, because it is oxygen sensitive. Vanadium(III) is more stable than vanadium(II); however it is also gradually oxidized by oxygen in air or by dissolved oxygen. Consequently, vanadium(IV) and vanadium(V) are considered to be dominant in the environment. Vanadium(V) compounds are appreciably more toxic than those of the other valences. However, there is no method of selectively determining vanadium(V) from the other valences. Therefore, the vanadium(V) reagent, which has been used in animal experiments to evaluate the toxicity of vanadium(V), possibly contains vanadium of different valences, especially vanadium(IV).

Some methods of vanadium determination are presently available including spectrophotometry, titrimetry, atomic absorption spectrometry, and inductively coupled plasma atomic-emission spectrometry (ICP-AES). Spectrophotometry and titrimetry are influenced by the chemical states of vanadium, making it necessary to control the valence of vanadium by oxidants or reductants. However, no method has yet been presented in which the dependence on the valences are utilized for speciation of vanadium in spectrophotometry or titrimetry. Atomic absorption spectrometry and ICP-AES cannot be used alone for speciation, because differences in the chemical states of any samples are lost upon atomization. High-performance liquid chromatography (HPLC) is widely used for selective determination of various chemical species, and this method is easy to execute. Some complexes of transition metals and chelating reagents have been separated by HPLC and detected by spectrophotometer. The valence of vanadium was controlled to five in those reports, however. Selective determination of vanadium(IV) and vanadium(V) using HPLC has not been achieved. Therefore, an easy method for speciation of vanadium is required.

As different-valence vanadium species may exist as ions of different charge density in aqueous solution, they may be separated by HPLC with ion-exchange columns. As ICP-AES determines elements selectively by spectroscopy, its signal intensity is not influenced by drastic changes of elution composition. ICP-AES equipment can also be connected directly to a chromatograph. Further, ICP-AES has high sensitivity compared with flame atomic absorption spectrometry, especially where vanadium is considered. The present study thus presents a method for speciation of vanadium(IV) and vanadium(V) using a combination of HPLC and ICP-AES as the detector.
EXPERIMENTAL

Equipment

The equipment used in the present study consisted of pump systems, an ion-exchange column, an ICP-AES, and supplementary devices such as an eluent degasser, a column oven, and an ICP-signal processor and so on. A block diagram of the equipment is shown in Fig. 1.

Samples were injected by a sample injector (7100 Reodine, Cotati), using a 100 µL sample loop. The temperature of the ion-exchange column was maintained at 40°C in a column oven (AO-50, Showa-denko, Tokyo). The flow from the ion-exchange column was directly introduced into the ICP-AES equipment (Plasma 40, Perkin-Elmer, Norwalk), and the vanadium in the eluent was determined. The strongest emission line of vanadium at 309.311 nm was measured. Wavelength adjustment and sensitivity calibration were performed before each measurement.

Emission-signal output (0-10V) from ICP-AES was passed through a low-pass-filter (6-BL-DC cutoff frequency 20 Hz, NF, Yokohama) to remove power line noise of 50 Hz. Amplified by 50 times, the signal was digitized by a 12-bit analog-digital converter unit (CHS-ADT-711, Canopus, Kobe), with a sampling rate of 1 kHz. The digitized signals were accumulated in a personal computer (PC-286LS, EPSON, Suwa), and a chromatogram of 10 Hz time resolution was obtained.

In the equipment, three types of pump systems were used, which corresponded to three procedures. These pumps are shown in Fig. 2a, 2b and 2c. Figure 2a shows a single eluent pump system, which was composed of a pump (LC-6A, Shimazu, Kyoto), an online-degasser (DG-2400, Uniflows, Tokyo), and 5 meters of SUS tube, the inner diameter of which was 0.1 mm. The SUS tube was connected to the outlet
of the pump to provide added pressure resistance. The pump is not designed to be connected to an ion-exchange column, which has a lower flow resistance than other types of HPLC columns. If directly connected to the pump, the ion-exchange columns would thus be damaged by pressure ripples from the pump. These pressure ripples was reduced significantly by the added pressure resistance of the SUS tube.

Figure 2b shows the gradient-elution system. Two types of eluents were mixed in any ratio by two flow-rate-programmable pumps (PU-980i, Nihon-bunko, Tokyo) and a high-pressure mixer (PU-980i-30, Nihon-bunko, Tokyo). These pumps could be connected to an ion-exchange column without pressure dampers.

Figure 2c shows a stepwise-elution system, which consisted of a single elution pump system (same as shown in Fig. 2a) and a three-way stop cock. Two eluents could be selected by the three-way stop cock.

Fig. 2a, 2b, 2c. Block diagrams of pump systems and elution diagrams.
Reagents

All reagents were purchased from Wako-Junyaku (Osaka) and used without further purification. Vanadium(IV) oxide sulfate trihydrate of 99.9% purity was used as the vanadium(IV) standard reagent. This purity meant that contamination by other elements was controlled to be less than 0.1%; the reagent was not warranted to be vanadium(V)-free. Ammonium metavanadate was used as the vanadium(V) standard reagent, and was not warranted to be vanadium(IV)-free. However, these reagents were taken as the pure standards for vanadium(IV) and vanadium(V) respectively in the present study.

Preparations of standard sample solutions

To obtain a vanadium(IV) 1,000 µg/mL standard sample solution, 426 mg of vanadium(IV) oxide sulfate trihydrate (VOSO₄ • 3H₂O) was dissolved in 100 mL of 1 mM nitric acid. The 1,000 µg/mL solution was diluted to final concentrations with 1 mM nitric acid.

To obtain a vanadium(V) 1,000 µg/mL standard sample solution, 229 mg of ammonium metavanadate (NH₄VO₃ • 3H₂O) was dissolved in about 100 mL of distilled water with ultrasonic agitation, and 1.1 mL of 1 M nitric acid was added slowly for pH adjustment. The solution was diluted to final concentrations with 1 mM nitric acid.

Preparations of eluents

Nitric acid (d = 1.38) was diluted to about 1 M with water. The concentration of the solution was determined by titration with 0.1 M sodium hydroxide standard solution. The ca. 1 M solution was further diluted to 100 mM and 1 mM.

Buffer solutions were prepared with tartric acid, benzoic acid or citric acid and potassium hydroxide or tris(hydroxymethyl)aminomethane. Acid concentration of all the buffer solutions were held constant at 4 mM, and pH levels were controlled by basic concentrations.

Procedures

In the present experiment, three procedures were examined. All samples were prefiltred by membrane filter (SJHV 13, pore size 0.45 µm, Nihon Millipore, Yonezawa). The flow rates of eluents during all procedures was 1.0 mL/min, a limitation imposed by the specifications of the ICP-AES (Plasma 40).

Procedure 1

A Shodex Y521 cation-exchange column (Showa-denko, Tokyo) was used. Eluent was 4 mM tartaric acid-tris(hydroxymethyl)aminomethane buffer solution of pH 4, which was the eluent recommended by the manufacturer. The pump system shown in Fig. 2a was used.
Procedure 2
A TSK-gel IC Cation cation-exchange column (Tohsoh, Tokyo) was used. Two eluents, 4 mM benzoic acid-potassium hydroxide buffer solution and 4 mM citric acid-potassium hydroxide buffer solution, both with pH 4, were used in gradient-elution by the pump system shown in Fig. 2b. At the beginning, benzoic acid buffer was used as the eluent, and a sample was injected into the system. Three minutes later, addition of citric acid buffer to the eluent was started. Five minutes after addition of citric acid buffer began, the eluent was changed completely to citric acid buffer. Ratio of the benzoic acid buffer to the citric acid buffer changed linearly over time. Data collection continued for 15 minutes from injection. After each measurement, a 15-minutes interval before the next injection was allowed for column reconditioning. During this reconditioning period, benzoic acid buffer was sent to the column. The elution diagram is shown in Fig. 2b.

Procedure 3
A Shodex 1524A anion-exchange column (Showa-denko) was used. One mM nitric acid and 100 mM nitric acid solutions were used as eluents in sequence with low-pressure stepwise-elution technique using the pump system shown in Fig. 2c. At the beginning, 1 mM nitric acid was used as the eluent. Five minutes after injection, the eluent was changed to 100 mM nitric acid by switching the flow route. One hundred mM nitric acid was then sent to column as eluent for 15 minutes. After measurement, 1 mM nitric acid was passed through the column as eluent for 20 minutes, in order to recondition it. The elution diagram is shown in Fig. 2c.

RESULTS AND DISCUSSION

Procedure 1
The chemical forms of vanadium(IV) and vanadium(V) vary depending on pH, as shown in Fig. 318). In the eluent of pH 4, vanadium(V) is expected to exist as oxoacid anions. This might be the reason that vanadium(V) passed through the cation-exchange column. On the other hand, vanadium(IV) cations were trapped on the cation-exchange column in this eluent, becoming separated from vanadium(V). However, the Y521 cation-exchange column is designed for the separation of single-valent cations, and vanadium(IV) cations (VO²⁺) were not eluted from Y521. Complexing reagents in eluent were expected to elute vanadium(IV) cations, and tartaric acid was tried to elute vanadium(IV) cations (VO²⁺) as complexing reagents in this procedure.

The retention time of vanadium(IV) in this procedure depended on its concentration (Fig. 4). The data shows that the complex of vanadium(IV) and tartaric acid is labile, and a part of vanadium(IV) was trapped on cation-exchange column. Therefore, this procedure proved not to be applicable to the separation of vanadium(IV) and vanadium(V).
Fig. 3. Vanadium species in each pH.  
The lines under each species show the ranges of pH in which the species predominantly exist.

<table>
<thead>
<tr>
<th>pH</th>
<th>$VO_2^+$</th>
<th>5 insoluble</th>
<th>8 $V_2O_5^{2-}$</th>
<th>$V(IV)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$VO_2^-$</td>
<td>$[H_4(V_2O_5)]^{3-}$</td>
<td>$V_2O_4^{4-}$</td>
<td>$V(V)$</td>
</tr>
<tr>
<td>2</td>
<td>$[V_2O_4]^0$</td>
<td>$[H_2(V_2O_13)]^{4-}$</td>
<td>$VO_4^{3-}$</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4. Chromatograms of V(IV) in procedure 1.  
Curve a is a chromatogram of V(IV) 20 µg/mL. Curve b is a chromatogram of V(IV) 2 µg/mL. The ordinate of curve b is magnified by 20 times relative to that of curve a.
Procedure 2

Citric acid is a stronger complexing reagent than tartaric acid, and completely eluted vanadium(IV) from the cation-exchange column. However, its retention time became zero, and the retention time of vanadium(V) oxoacid anion was also zero. Therefore, citric acid alone could not be used to separate vanadium(IV) and vanadium(V).

When benzoic acid buffer solution of pH 4 was used as an eluent, which has no complexing ability, vanadium(IV) cation was trapped on the cation-exchange column, while vanadium(V) oxoacid anion passed through the column. After this separation, citric acid buffer of pH 4 eluted vanadium(IV) as citrate complex anions from the cation-exchange column. The chromatograms of this procedure are shown in Fig. 5.

In this condition, a part of the vanadium(V) oxoacid anions broke down into free cations, and these became trapped on cation-exchange column. The ratio of the trapped to the passing fraction depended on the concentration of vanadium(V). Therefore, this procedure proved not to be applicable to selective determination of vanadium(IV) and vanadium(V).
**Procedure 3**

Vanadium(V) takes the form of oxoacid anions in a solution of pH 3, which become cations in a solution of pH 1. So vanadium(V) was expected to be trapped on an anion-exchange column with pH 3 eluent, and to be eluted from the column with pH 1 eluent. In the first step of the two step elution, 1 mM nitric acid was used as a pH 3 eluent. Vanadium(IV) cation passed through a 1524 anion-exchange column with a retention time of zero, while vanadium(V) oxoacid anion was trapped on the column. When the eluent was switched to 100 mM nitric acid (pH 1) after this separation, vanadium(V) cations were eluted. The chromatograms of this procedure are shown in Fig. 6.

A pair of peaks due to vanadium(IV) were observed at pH 3 and pH 1 (P$_3^{IV}$ and P$_1^{IV}$, respectively). Another pair of peaks due to vanadium(V) were observed at pH 3 and pH 1 (P$_3^{V}$ and P$_1^{V}$, respectively). Peaks P$_3^{IV}$ and P$_1^{IV}$ are attributed to vanadium(IV) cation and vanadium(V) cation trapped on the column as oxoacid anion in the pH 3 solution, respectively. However, the attribution of P$_1^{IV}$ and P$_3^{V}$ is not...
When sample solutions prepared about one month before the determination were used, \( P_{1IV} \) was larger than those of the fresh samples. When nitric acid solutions were bubbled with pure nitrogen to purge dissolved oxygen, and were used for diluting standard sample solutions, \( P_{1IV} \) was reduced significantly. Therefore a part of \( P_{1IV} \) was regarded as vanadium(V), which was produced by oxidation of vanadium(IV) by dissolved oxygen. However, addition of reductants such as hydroquinone or hydroxylammonium chloride to the sample solutions could not eliminate \( P_{1IV} \) completely. The \( P_{1IV} \) of the reductant-treated sample were nearly equal to the \( P_{1IV} \) of fresh samples which contained dissolved oxygen (Fig. 7). All of \( P_{1IV} \) is not attributed to vanadium(V) produced by oxidation of vanadium(IV) or contained as an impurities in vanadium(IV). This shows the presence of trace vanadium(IV) oxoacid anion in weak acidic conditions.

A part of vanadium(V) which passed through the anion-exchange column at pH 3 (\( P_{3V} \)) was expected from the known vanadium states at each pH, as shown in Fig. 3. The ratio of \( P_{3V}/P_{1V} \) was independent of the concentration of vanadium(V).
This two-step eluent method was examined for eluent at various pH values to find the optimum condition. The tailing of the second peak of vanadium(V) was eliminated by changing the second-step eluent to a stronger acidic solution such as pH 0. However, the acidic limit of the column is pH 1, hence the second-step eluent could not be changed.

The chromatogram with a pH 4 buffer solution as the first-step eluent is shown in Fig. 8. The escaping fraction of vanadium(V) at the first-step was quite reduced. However the peak of vanadium(IV) passing through the column (P₄IV) with this eluent was broadened, and hence the determination limit of vanadium(IV) was lower than when a pH 3 eluent was used for as the first elution. When a pH 3 nitric acid solution was used as the first eluent, 15% of vanadium(V) passed through the column, and 7% of vanadium(IV) was trapped on the column. The concentrations of vanadium(IV) and vanadium(V) were determined by the calculation described below. It exhibited higher sensitivity than using pH 4 buffer, so a pH 3 nitric acid solution was used as the first step eluent. Therefore the foregoing condition was determined as optimum.
Calculation

Peak areas (P₁⁴⁺, P₂⁴⁺, P₃⁴⁺, P₄⁴⁺) of vanadium(IV) and vanadium(V) varied with their concentrations as shown in Fig. 9. The area of each peak was proportional to the sample concentration. Four linear equations, showing the relations between the sample concentrations of vanadium and the peak areas, were obtained by the least square method.

The equations were as follows:

\[
P₁⁺⁴⁺ = 0.694 \times C⁺⁴⁺ - 0.001 \quad r = 0.999 \quad (1a)
\]

\[
P₂⁺⁴⁺ = 0.069 \times C⁺⁴⁺ + 0.034 \quad r = 0.782 \quad (1b)
\]

\[
P₁⁺⁵⁺ = 0.149 \times C⁺⁵⁺ + 0.0137 \quad r = 0.968 \quad (1c)
\]

\[
P₂⁺⁵⁺ = 0.916 \times C⁺⁵⁺ + 0.028 \quad r = 0.993 \quad (1d)
\]

where

C⁺⁴⁺: concentration of vanadium(IV)
C⁺⁵⁺: concentration of vanadium(V)

The following two equations were derived from equation (1a) + equation (1c) and equation (1b) + equation (1d).

\[
P₁⁺⁴⁺ + P₁⁺⁵⁺ = 0.694 \times C⁺⁴⁺ + 0.149 \times C⁺⁵⁺ + 0.136 \quad (2a)
\]

\[
P₂⁺⁴⁺ + P₂⁺⁵⁺ = 0.069 \times C⁺⁴⁺ + 0.916 \times C⁺⁵⁺ + 0.062 \quad (2b)
\]

Concentrations of vanadium(IV) and vanadium(V) in the mixed solutions of known concentrations of vanadium(IV) and vanadium(V) were determined by using the
equations (2a) and (2b). Standard solutions of vanadium(IV) and vanadium(V) were mixed in the ratios of vanadium(IV) to vanadium(V) of 2:8, 5:5 and 8:2, with a total vanadium concentration of 10 μg/mL. These mixed samples were examined by procedure 3, and the areas of the two peaks (P3mixed, P1mixed) were obtained for each sample. Two simultaneous linear equations were derived from these values, where P3IV + P3V was substituted for P3mixed in equation 2a, and P1IV + P1V was substituted for P1mixed in equation 2b. Each concentration of vanadium(IV) and vanadium(V) was determined by solving these equations. A comparison of the prepared concentrations and determined concentrations obtained by a combination of analysis and calculation is shown in Table 1.

### CONCLUSIONS

Vanadium ions behave very complexly in aqueous solutions, and hence the conventional ion-exchange HPLC technique could not be used to speciate them. Procedure 3, in which a two-step elution method and a basic calculation technique were combined and simultaneous linear equations solved, proved capable of determining vanadium(IV) and vanadium(V). The determination limit of this method was 1 μg/mL which depended on the LOD of ICP-AES. Japanese regulations limit vanadium concentrations in workroom air to 0.03 mg/m³ as vanadium. The conventional sampling method of vanadium pentoxide, which is recommended by Japanese Ministry to Labour, is as follows.

Samples should be taken by filtration with a membrane filter. The sampling volume is 0.1 m³ (10 L/minute, 10 minutes). The membrane filter is decomposed by acid digestion and the residue is dissolved in 50 mL of water. Therefore, the 0.03 mg/m³ limit corresponds to 0.06 μg/mL in aqueous solution.

The sensitivity of the present method is not satisfactory to speciate the vanadium compounds in a work environment with conventional sampling and dissolution conditions. If the sampling time is elongated to 90 min and filtered dust is dissolved

### Table 1. Prepared concentrations vs. analyzed concentrations of vanadium(IV) and vanadium(V)

<table>
<thead>
<tr>
<th>Testing solution</th>
<th>Prepared concentrations [μg/mL]</th>
<th>Analyzed concentrations [μg/mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vanadium(IV)</td>
<td>Vanadium(V)</td>
</tr>
<tr>
<td># 1</td>
<td>0.2001</td>
<td>0.7781</td>
</tr>
<tr>
<td># 2</td>
<td>0.2001</td>
<td>0.7781</td>
</tr>
<tr>
<td># 3</td>
<td>0.5287</td>
<td>0.4832</td>
</tr>
<tr>
<td># 4</td>
<td>0.5287</td>
<td>0.4832</td>
</tr>
<tr>
<td># 5</td>
<td>0.8435</td>
<td>0.1925</td>
</tr>
<tr>
<td># 6</td>
<td>0.8435</td>
<td>0.1925</td>
</tr>
</tbody>
</table>
into 20 to 30 ml of an adequate acid solution, the LOD reaches to the regulatory limit of 0.03 mg/m³.

Other than assessment of workplace contamination, selective determination of vanadium species serves some other important purposes, such as characterization of exposure reagents used in animal toxicology experiments. At present there is no easy method for speciation of vanadium species. Consequently vanadium(V) reagents, which are not warranted to be vanadium(IV)-free, have been used in animal exposure experiments. When such non-warranted vanadium(V) reagent was used, there is a probability that vanadium(V) toxicity has been underestimated. The present method thus provides a very useful means of checking for impurities, in the form of vanadium of the other valent states, in vanadium(IV) reagents and/or vanadium(V) reagent.

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