Isotope Dilution Analysis of Se in Human Blood Serum by Using High-Power Nitrogen Microwave-Induced Plasma Mass Spectrometry Coupled with a Hydride Generation Technique

Masaki Ohata, Tatsuya Ichinose, and Naoki Furuta*

Faculty of Science and Engineering, Department of Applied Chemistry, Chuo University, 1-13-27, Kasuga, Bunkyou-ku, Tokyo 112-8551, Japan

Atsuko Shinohara and Momoko Chiba

Department of Epidemiology and Environmental Health, Department of Internal Medicine, Juntendo University School of Medicine, 2-1-1, Hongo, Bunkyou-ku, Tokyo 113-0033, Japan

To establish a method for sensitive, accurate, and precise determination of Se in real samples, isotope dilution analysis using high-power nitrogen microwave-induced plasma mass spectrometry (N2 MIP-IDMS) was conducted. In this study, freeze-dried human blood serum (Standard Reference Material, NIES No. 4) provided by NIES (National Institute for Environmental Studies) was used as a real sample. The measured isotopes of Se were $^{78}$Se and $^{80}$Se which are the major isotopes of Se. The appropriate amount of Se spike solution was theoretically calculated by using an error multiplication factor (F) and was confirmed experimentally for the isotope dilution analysis. The mass discrimination effect was corrected by using a standard Se solution for the measurement of Se isotope ratios in the spiked sample. However, the sensitivity for the detection of Se was not so good and the precision of the determination was not improved (2–3%) by N2 MIP-IDMS with use of the conventional nebulizer. Therefore, a hydride generation system was connected to N2 MIP-IDMS as a sample introduction system (HG-N2 MIP-IDMS) in order to establish a more sensitive detection and a more precise determination of Se. A detection limit ($3\sigma$) of 10 pg mL$^{-1}$ could be achieved, and the RSD was less than 1% at the concentration level of 5.0–10.0 ng mL$^{-1}$ by HG-N2 MIP-IDMS. The analytical results were found to be in a good agreement with those obtained by the standard addition method using conventional Ar ICP-MS.

It is well-known that Se is an essential element for all mammals. Se deficiency leads to deficiency syndromes, for example, Keshan disease, which is known for cardiac insufficiency that occurred in children and pregnant women in China. Problems also occur if the concentration of Se is too high; for example, gastroenteric disorders, dermatitis, and neurotic disorders are caused by excessive intake of Se. Moreover, it is well-known that the range of permissive intake amounts of Se is very narrow for human beings. Therefore, it is restricted as a toxic element in environmental standards. There are several sources of environmental Se pollution: the processes of Se refinement and the production processes of Se-containing products. For these reasons, the accurate and precise determination of trace levels of Se in environmental and biological samples is required, and studies of Se determination have been reported by several groups.1–11 Because Ar ICP-MS can measure multiple elements at a concentration range from ng mL$^{-1}$ to fg mL$^{-1}$, it has widespread use in the determination of trace elements in various samples.12–25 However, it has widespread use in the determination of trace elements in various samples.
spectral interference is still a serious problem. The polyatomic ions caused by the plasma-sustained gas of Ar, such as $^{40}$Ar$^{12}$C$^+$, $^{40}$Ar$^{16}$O$^+$, $^{38}$Ar$^{35}$Cl$^+$, $^{38}$Ar$^{40}$Ar$^+$, and $^{40}$Ar$^{40}$Ar$^+$, interfere the determination of $^{52}$Cr$^+$, $^{39}$Fe$^+$, $^{75}$As$^+$, $^{78}$Se$^+$, and $^{80}$Se$^+$, respectively. To reduce the Ar-associated polyatomic ions, ICP sources that are maintained by gases other than Ar$^{22,28}$ or mixed gases with Ar$^{29,30}$ and several types of MIP sources$^{31-36}$ have been developed and studied. A high-power $N_2$ MIP-MS constructed by Hitachi Co. (Ibaraki, Japan) is one of these sources which can be sustained by nitrogen gas$^{37,38}$. Because the interference of Ar-associated polyatomic ions is replaced with nitrogen-related polyatomic ions such as $^{14}$N$^+$, $^{15}$N$^+$, and $^{14}$N$^{18}$O$^+$, the high-power $N_2$ MIP-MS can determine the elements that are mentioned above without spectral interference$^{38,39}$. However, the detection sensitivity of $N_2$ MIP-MS is about 1 order of magnitude lower than that of Ar ICPMS for the elements with ionization energy more than about 8 eV, such as As and Se, because the temperature of the $N_2$ MIP is lower than that of the Ar ICP$^{40}$. The detection limit of Se was 78 pg mL$^{-1}$ by $N_2$ MIP-MS with use of the conventional nebulizer$^{41}$.

The purpose of this study is to improve sensitivity, accuracy, and precision for the determination of low concentration levels of Se (about 1–10 ng mL$^{-1}$). The isotope dilution analysis (ID) was adopted in this study because the sample loss during digestion procedures does not influence the determination of Se, and the accuracy and the precision of quantitation are better than those of other conventional methods such as the calibration method and the standard addition method. At first, Se determination was carried out by $N_2$ MIP-IDMS with use of the conventional nebulizer. However, the precision of the determination was almost the same as the conventional methods because the signal intensities of Se were not sufficiently high. In the next step, to achieve high sensitivity, a hydride generation technique (HG) was adopted as a sample introduction system. The hydride generation technique is very effective not only for high sensitivity but also for elimination of matrix elements$^{7,11}$. In this study, Se in freeze-dried human blood serum was determined by $N_2$ MIP-IDMS and HG-$N_2$ MIP-IDMS. The results obtained in this study are compared with those of other conventional methods and discussed in detail.

**EXPERIMENTAL SECTION**

**Instruments**. The $N_2$ MIP-MS used for this study was a P-7000 instrument that was commercially available from Hitachi Co. (Ibaraki, Japan). At first, the isotope dilution analysis of Se was conducted with the conventional nebulizer. The conditions followed those of ref 42. Furthermore, in this study $H_2$ MIP-IDMS was developed for the determination of Se. The schematic diagram of the instrument for $N_2$ MIP-IDMS is shown in Figure 1. The hydride generation system used was provided by Cetac (Omaha, NE). The sample solution of 21 mL is merged with hydrochloric acid (HCl) and sodium tetrahydroborate (NaBH$_4$) in a sodium hydroxide (NaOH) solution by a peristaltic pump, and these solutions are mixed in the reaction coil (i.d. 1 mm, 50 cm long) at room temperature (25°C). After that, the generated hydride of Se and other solutions are separated in the gas–liquid separator. The separated hydride of Se is introduced continuously into the $N_2$ MIP source by the carrier gas. The operating conditions of HG-$N_2$ MIP-IDMS are listed in Table 2. The operating conditions were optimized for both the hydride generation system and the $N_2$ MIP-IDMS measurement system.

**Reagents**. A standard solution of Se(IV) (1000 µg mL$^{-1}$) was purchased from Kanto Chemical Co. (Tokyo, Japan). An appropriate concentration of Se(IV) was prepared by diluting with 0.1 mol L$^{-1}$ HNO$_3$. The 0.1 mol L$^{-1}$ HNO$_3$ was prepared by diluting high-purity grade 70%HNO$_3$ (Kanto Chemical Co., Tokyo, Japan).

(22) Hwang, T.-J.; Jiang, S.-J.; Analyst 1997, 12, 233–237.

![Schematic diagram of HG-$N_2$ MIP-IDMS](image-url)

Table 1. Operating Conditions of $N_2$ MIP-MS for Isotope Dilution Analysis ($N_2$ MIP-IDMS)

<table>
<thead>
<tr>
<th>$N_2$ MIP-MS</th>
<th>frequency</th>
<th>2.45 GHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>microwave power</td>
<td>1.3 kW</td>
<td></td>
</tr>
<tr>
<td>plasma gas flow rate</td>
<td>15.0 L min$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>carrier gas flow rate</td>
<td>1.3 L min$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>peak point/ mass</td>
<td>8 point</td>
<td></td>
</tr>
<tr>
<td>dwell time/ point</td>
<td>1.0 ms</td>
<td></td>
</tr>
<tr>
<td>number of sweeps</td>
<td>1500 times</td>
<td></td>
</tr>
<tr>
<td>repetition of sample and blank</td>
<td>5 times</td>
<td></td>
</tr>
</tbody>
</table>
isotopic abundance of the Se standard solution was considered 
atomic weights of Se in both natural and spike solutions. The 
spike solution of Se used was purchased from 
Teknolab A/S (Norway). Table 3 shows isotopic abundances and 
grade. The spike solution of Se used was purchased from 
Japan) with Milli-Q purified water (Milli-Q SP ICPMS, M illipore, 
Tokyo, Japan). HNO₃ and H₂O₂ used for digestion procedures 
and HCl used for the reduction from Se(VI) to Se(IV) were EL 
grade (Kanto Chemical Co., Tokyo, Japan). NaOH and NaBH₄ 
that were used for a hydride generation technique were reagent 
grade. The spike solution of Se used was purchased from 
Teknolab A/S (Norway). Table 3 shows isotopic abundances and 
atomic weights of Se in both natural and spike solutions. The 
iso-

topic abundance of the Se standard solution was considered 
to a natural isotopic abundance that was reported by IUPAC (International Union of Pure and Applied Chemistry) in 1991.43,44

Sample Preparation. Freeze-dried human blood serum 
(SRM NIES No. 4) was obtained from NIES (National Institute 
for Environmental Studies, Tsukuba, Japan). The reference value 
of Se is 0.14 μg mL⁻¹ when the serum is dissolved in 5 mL of 
water, but it is not a certified value. After the serum in a vial was 
transferred to a Teflon beaker, an appropriate amount of the Se 
spike solution was added. To dissolve the serum, 8 mL of 70% 
HNO₃ was added and the resulting solution was soaked overnight. 
After that, 2 mL of 70%HNO₃ and 3 mL of 30%H₂O₂ were added 
and heated on a hot plate at 70–80 °C for 10 h. It is considered 
practically that loss of Se occurs when the heating temperature is too high, because selenium is easily vaporized as a form of 
al- 

hydrides.7

Furthermore, 2 mL of 70%HNO₃ and 3 mL of 30%H₂O₂ were added 
and heated until the sample solution became transparent. The serum was then diluted to 50 mL, that is, the serum was diluted by a factor of 10 using Milli-Q water. This sample solution was used for the nebulizer method. At this stage, the concentration of Se was about 14 ng mL⁻¹. In this study, the hydride 
generation was also used as a sample introduction technique. For 
the determination of total Se in the serum, Se(VI) has to be 
reduced to Se(IV) because Se(IV) does not generate selenium hydrides.7–11,45 
The complete and quantitative reduction from Se(VI) to Se(IV) was carried out easily and quickly by the method described in ref 45. An 11 mL amount of 35%HCl was added to 
10 mL of the digested sample, the acidity was adjusted to 6 mol 
L⁻¹, and the reduction from Se(VI) to Se(IV) was carried out by

7 min of soaking in a water bath at 70 °C.45 Therefore, the serum 
had been diluted by a factor of about 20, finally. The final 
concentration of Se was approximately 7 ng mL⁻¹.

Correction for the Mass Discrimination Effect. It is well-
known that the mass discrimination effect must be corrected for 
to obtain accurate ratio values. The mass discrimination correction 
was carried out with a standard Se solution. The correction 
was achieved by using eqs 1 and 2.

\[
\frac{2.086}{R_{\text{standard}}} = E \quad (1) \\
R_{\text{spiked}}E = R_{\text{true}} \quad (2)
\]

where E is a correction factor of the mass discrimination effect, 
Rstandard is the ratio value of 78Se/80Se in a Se standard solution 
without a spike, Rspiked is the resultant ratio value of 78Se/80Se in 
a sample with a spike, and Rtrue is the corrected isotope ratio that 
is used for the isotope dilution analysis. The concentration of Se 
in standard solutions used for the correction of the mass 
discrimination effect was 100 ng mL⁻¹ for N₂ MIP-IDMS with 
use of the nebulizer and 10 ng mL⁻¹ for HG-N₂ MIP-IDMS. 
These concentration levels of Se in standard solutions are 
adequate to determine the Rstandard values that are attributed to 
the instrumental characteristics.

RESULTS AND DISCUSSIONS
Optimization of the Amount of a Se Spike Solution. The 
isotope dilution analysis is a technique for the determination of 
analyte in a sample by measuring a resultant ratio of the analyte 
after addition of a spike whose isotope abundance differs greatly 
from the natural abundance of the analyte.12–22 Once the isotopic 
equilibrium is reached, the analyte loss during digestion pro-
duces does not influence the analytical result. That is one of big 
advantages of the isotope dilution analysis. The concentration 
of Se can be calculated by eq 3.

\[
C = \frac{W_{\text{sp}}(A_{\text{sp}} - RB_{\text{sp}})}{W_{\text{s}}M_{\text{sp}}(RB_{\text{t}} - A_{\text{t}})} \quad (3)
\]

where C is the concentration of Se in the sample (ng mL⁻¹), Wsp 
is the weight of Se in the spike (ng), Ws is the total amount of the 
serum sample (mL), M is the atomic weight of Se (78.96) in the 
spike, Ms is the atomic weight of Se (78.02) in the spike, A sp 
and Bsp are the abundances of 78Se (23.78%) and 80Se (49.61%) in 
the sample, respectively, Ap and B are the abundances of 78Se 
(98.58%) and 80Se (1.00%) in the spike, and R is the Se isotope 
ratio (78Se/80Se) measured experimentally. The resultant ratio R 
was obtained after subtracting ion counts of the laboratory reagent 
blank obtained just before the sample measurement. Evaluation 
of a spike amount has already been reported by several groups.12–14

It is well-known that a spike amount influences the precision of 
determinations, which is referred to as an error multiplication 
factor (F). The precision of determinations (\sigma/dC) is always 
lower than that of isotope ratio measurements (\sigma/R/I) and is 
obtained by multiplying the precision of isotope ratio measure-
ments by the factor F (eq 4). The F values can be calculated 
theoretically with eq 5.12 From eq 5, it is evaluated that better
precision can be obtained when a spike solution is added to the sample so as to make the F value closer to 1, by assuming the precision of isotope ratio measurements (dR/R) is constant. In this study, we evaluated the effect of the amount of the Se spike on the precision of the determinations. The theoretical F values for various Se (78Se/80Se) isotope ratios were calculated by eq 5 and are shown in Figure 2. The F values obtained experimentally are also plotted in Figure 2. These results were obtained by N2 MIP-IDMS with use of the nebulizer. The measured isotope ratios were 0.9, 2.4, 7.9, 21, and 54. The experimentally obtained F values were in good agreement with the theoretical values. From Figure 2 it can be evaluated that the best precision is obtained when the resultant ratio is about 8. The same experiment was conducted with use of hydride generation. The result was the same as that with use of the nebulizer. Therefore, an appropriate amount of the Se spike solution was added so as to make the resultant ratio of Se about 8.

**Determination of Se in Human Blood Serum by Using N2 MIP-IDMS and HG-N2 MIP-IDMS.** The determination of Se in freeze-dried human blood serum (SRM NIES No. 4) was conducted by N2 MIP-IDMS with use of the conventional nebulizer and by HG-N2 MIP-IDMS. The analytical results of Se in human blood serum are summarized in Table 4. An appropriate amount of the spike solution was added to each sample for the isotope dilution analysis as mentioned in the previous section, and three spiked samples (spikes A, B, and C in Table 4) were measured in this study. As mentioned in the Experimental Section, this serum sample was diluted by a factor of 10 for N2 MIP-IDMS with use of the conventional nebulizer and by a factor of 20 for HG-N2 MIP-IDMS. Therefore, the expected concentrations of Se were about 0.9, 2.4, 7.9, 21, and 54. The measured isotope ratios are in Table 4. The brackets indicate RSD (%), which was calculated with five determinations of Se (78Se/80Se) isotope ratios were calculated by eq 5 and are shown in Figure 2. The F values obtained experimentally are also plotted in Figure 2. These results were obtained by N2 MIP-IDMS with use of the nebulizer. The measured isotope ratios were 0.9, 2.4, 7.9, 21, and 54. The experimentally obtained F values were in good agreement with the theoretical values. From Figure 2 it can be evaluated that the best precision is obtained when the resultant ratio is about 8. The same experiment was conducted with use of hydride generation. The result was the same as that with use of the nebulizer. Therefore, an appropriate amount of the Se spike solution was added so as to make the resultant ratio of Se about 8.

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**Table 3. Relative Isotopic Abundance (atom %) and Atomic Weight (g mol⁻¹) of Se in Both Natural and Spike Solutions**

<table>
<thead>
<tr>
<th>mass number</th>
<th>natural</th>
<th>spike</th>
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<tbody>
<tr>
<td>74</td>
<td>0.89</td>
<td>0.06</td>
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<tr>
<td>76</td>
<td>9.36</td>
<td>0.11</td>
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<td>77</td>
<td>7.63</td>
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<td>78</td>
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<td>80</td>
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<td>1.00</td>
</tr>
<tr>
<td>82</td>
<td>8.73</td>
<td>0.08</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>atomic weight</th>
<th>reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>78.96</td>
<td>Se standard solution (Kanto Chemical)</td>
</tr>
<tr>
<td>78.02</td>
<td>Spectrascan (Teknolab A/S)</td>
</tr>
</tbody>
</table>

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**Figure 2.** Error multiplication factor (F) as a function of Se isotope ratio (78Se/80Se) after addition of a Se spike solution. The solid line shows a theoretical F curve, and ● indicates experimentally obtained F values.

**Table 4. Determination of Se (ng mL⁻¹) in Freeze-Dried Human Blood Serum (SRM NIES No. 4)**

<table>
<thead>
<tr>
<th>sample (resultant ratio of 78Se/80Se)</th>
<th>isotopic dilution analysis</th>
<th>standard addition method by Ar ICPMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>spike A (7.8)</td>
<td>127.4 ± 0.8 [0.6]</td>
<td>123.8 ± 3.8 [3.0]</td>
</tr>
<tr>
<td>spike B (8.0)</td>
<td>128.8 ± 1.0 [0.8]</td>
<td>128.6 ± 2.3 [1.8]</td>
</tr>
<tr>
<td>spike C (8.4)</td>
<td>128.4 ± 0.7 [0.5]</td>
<td>125.5 ± 3.3 [2.6]</td>
</tr>
<tr>
<td>average</td>
<td>128.2 ± 0.8 [0.6]</td>
<td>126.0 ± 3.1 [2.5]</td>
</tr>
</tbody>
</table>

* The brackets indicate RSD (%), which was calculated with five measurements.

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**Methods.** The range of permissive intake amounts of Se is very narrow for human beings, so the accurate and precise determination of trace levels of Se is very important and is required. The improvement of the precision achieved in this study will contribute to the investigation of Se effects on human disease and the Se pollution in environment.

**CONCLUSIONS**

To achieve the sensitive, accurate, and precise determination of Se in human blood serum, the isotope dilution analysis using high-power nitrogen MIP-MS with a conventional nebulizer method and a hydride generation technique as a sample introduction system were conducted. Because the N2 MIP-MS does not have spectral interferences for the Se isotopes, the major isotopes of 78Se and 80Se could be used for the isotope dilution analysis. In the case of the isotope dilution analysis, the analyte loss during...
sample preparation does not influence the analytical result after an isotopic equilibrium is reached. The amount of a Se spike solution was optimized for the precise determination of Se using an error multiplication factor (F). With use of the hydride generation technique, the sensitivity was improved and the detection limit (3σ) of 10 pg mL⁻¹ could be achieved. The RSD was less than 1% at the concentration level of 5.0–10.0 ng mL⁻¹. It should be noticed that the correction of the mass discrimination effect was necessary to get accurate results. The analytical results of Se using the developed methods were in a good agreement with those obtained by the conventional standard addition method.

Then, it could be evaluated that the HG-N₂ MIP-IDMS method can utilize accurate and precise determination of Se in prepared solutions at the concentration level of more than 5.0 ng mL⁻¹.

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