An Application of Nitrogen Microwave-Induced Plasma Mass Spectrometry to Isotope Dilution Analysis of Selenium in Marine Organisms

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SHIRASAKI, T., YOSHINAGA, J., MORITA, M., OKUMOTO, T. and OISHI, K. *An Application of Nitrogen Microwave-Induced Plasma Mass Spectrometry to Isotope Dilution Analysis of Selenium in Marine Organisms.* Tohoku J. Exp. Med., 1996, 178 (1), 81-90 — Nitrogen microwave-induced plasma mass spectrometry was studied for its applicability to the isotope dilution analysis of selenium in biological samples. Spectroscopic interference by calcium, which is present in high concentrations in biological samples, was investigated. No detectable background spectrum was observed for the major selenium isotopes of ^{78}Se and ^{80}Se . No detectable interferences by sodium, potassium, calcium and phosphorus on the isotope ratio $^{80}\text{Se}/^{78}\text{Se}$ were observed up to concentration of 200 mg/ml. The method was applied to the analysis of selenium in biological reference materials of marine organisms. The results showed good agreement between the certified and found values. ————— trace element analysis; microwave-induced plasma; isotope dilution analysis; mass spectrometry

Selenium is an important element from both nutritional and toxicological viewpoints. However, among the many trace elements contained in biological samples, selenium has been a difficult one to precisely determine by spectrophotometric methods such as atomic absorption and atomic emission photometries. The wavelength of the atomic line of selenium used for atomic emission and absorption photometry is located in close vicinity to the vacuum ultraviolet region, where the transmittance of a light beam decreases steeply and results in a low signal-to-noise ratio with the spectrophotometers.

Recently, plasma mass spectrometry has been widely used for elemental analysis because of its capability for multielement analysis and low detection limits (Beauchemin et al. 1987; Suzuki et al. 1991; Sen Gupta 1993). In addi-

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tion, isotope dilution analysis may be the most reliable method for achieving a precise and accurate determination of trace elements in solution samples (Adams et al. 1988; Okamoto, K. 1991). This method is considered to be relatively free from interference effects by concomitants elements.

Although the use of inductively coupled plasma mass spectrometry (ICP-MS) for isotope dilution analysis has been widely used, ICP-MS is sometimes suffers from the formation of molecular ions originating from argon (^{36}Ar , ^{38}Ar and ^{40}Ar) in the mass number (m/z) range $m/z=50$ and 80 (Tan and Horlick 1986; Satzger et al. 1987). For ICP-MS, selenium is one such example because the mass number of selenium isotopes ^{80}Se , ^{78}Se , ^{77}Se and ^{76}Se are coincide with molecular ions originating from argon isotopes. The major isotopes of selenium $m/z=76$ (23.6%) and 80 (49.7%) are subject to severe interference from $^{40}\text{Ar}^{36}\text{Ar}$, $^{38}\text{Ar}^{38}\text{Ar}$ and $^{80}\text{Ar}_2$. In addition, other isotopes of lower abundance are also subject to molecular interference from halogens.

Shen et al. (1990a, b) demonstrated that nitrogen microwave-induced plasma mass spectrometry (MIP-MS) showed no spectroscopic interference in the m/z region of selenium isotopes. They employed a moderate power (500 W) nitrogen microwave-induced plasma. As a result, the system had a lower sensitivity compared with that of ICP-MS. Recently, Yoshinaga et al. (1995) reported an application of high power (1.3 kW) nitrogen MIP-MS to isotope dilution analysis of selenium in clinical samples. They showed that the high power nitrogen MIP-MS had a high sensitivity and low detection limit that was comparable with that of ICP-MS. In a previous report, (Yoshinaga et al. 1995), spectroscopic interferences at $m/z=80$ arising from SO_3^+ were studied.

In this report, interferences from sodium, potassium, calcium and phosphorus, which are present in marine organisms in high concentrations, were studied in an isotope dilution analysis of selenium with the high power nitrogen MIP-MS. In addition, to further ascertain the applicability of isotope dilution MIP-MS analysis of selenium to other biological matrices, reference materials of marine organisms were analyzed.

EXPERIMENTAL

Microwave-induced plasma

Microwave power (2.45 GHz, 1.3 kW) was fed to a cavity, which was cooled by circulating water at 20°C. A stable impedance matching was established between the microwave power source and the discharge plasma. The discharge was kept stable and was not influenced by the abrupt mixing of air into the nitrogen carrier gas while exchanging the sample solution (Okamoto, Y. 1991). A cross-sectional view of the cavity is shown in Fig. 1. A quartz discharge tube was inserted in the center of the cavity. A plasma support gas was fed through the cylindrical gap between two quartz tubes and a carrier gas was fed through the central orifice (i.d. 1.0 mm) of the quartz tube.

Fig. 1. Cross sectional view of a cavity where the nitrogen microwave-induced plasma was produced.

Mass spectrometer

An MIP mass spectrometer (P-6000; Hitachi, Ltd., Hitachinaka) was used in this study. A detailed description of the instrument is given by Oishi et al. (1994) and Okamoto (1994). Typical operating conditions used in this study for MIP-MS are shown in Table 1.

Sample introduction

A concentric type pneumatic nebulizer was used. The aqueous sample solution was aspirated at a rate of 0.25 liter/min by controlling the flow rate of the

TABLE 1. *Operating conditions*

Incident power	1.3 kW
Reflected power	< 10 W
Sampling cone	0.8 mm ϕ , orifice (Pt)
Skimmer cone	0.4 mm ϕ , orifice (Cu)
Plasma support gas flow	13 liter/min (nitrogen)
Carrier gas flow	1.3 liter/min (nitrogen)
Nebulizer	Pneumatic, concentric type
Temperature of spray chamber	5°C
Sample uptake rate	0.25 mm liter/min

nitrogen carrier gas (1.1–1.3 liter/min).

Reagents

Milli-Q purified water (18 M Ω) was used. Ultra pure nitric acid and hydrofluoric acid were purchased from Kanto Chemical Co., Ltd. (Tokyo). ^{78}Se (metal powder form) was purchased from Oak Ridge National Laboratory (Oak Ridge, TN, USA). A 25 mg portion of it was dissolved with concentrated nitric acid and diluted to 500 $\mu\text{g/g}$ with distilled water. A selenium standard solution of 50 $\mu\text{g/liter}$ prepared from a commercial 1,000 mg/liter standard for atomic absorption spectrophotometry (Kanto Chemical Co., Ltd., Tokyo) was also used for the optimization of the mass scanning condition. Suprapur grade sodium nitrate, potassium nitrate and calcium nitrate (Merck, Darmstadt, Germany) were dissolved in the distilled water to make 10 mg/ml solutions.

Sample preparation

Approximately 100 mg biological reference materials (NRCC TORT-1 lobster tomalley, NRCC DOLT-1 dogfish liver, NRCC DORM-1 dogfish muscle, IAEA Fish and NIES No. 6 Mussel) were accurately weighed into a PFA vial.

The optimum amount of the ^{78}Se spike solution (500 $\mu\text{g/g}$) was then added to place the $^{80}\text{Se}/^{78}\text{Se}$ ratio of the spike samples in the range 0.5–1. The samples were then digested with 2 ml of concentrated nitric acid at 140°C for 6 hr. After the samples were cooled, 0.1 ml hydrofluoric acid was added, and the mixture was heated at 140°C on a hot plate to dryness. After filteratin through a 0.45 μm membrane filter, the filtrate were dissolved with 0.1 ml nitric acid and made up to 5 ml with distilled water. An analytical blank was prepared in the same manner.

Mass scanning condition

The mass scanning conditions of the instrument that gave the optimum reproducibility for the isotope ratio measurements were selected as shown in Table 2. Data acquisition was selected as 1 point/mass and mass scanning was done by the peak jump mode. The measured isotope numbers were $m/z=78$ and 80. Ion counts at $m/z=78$ and 80 for the spiked sample solutions and the blank solution were measured. The values of $^{80}\text{Se}/^{78}\text{Se}$ for the samples were obtained after subtracting ion counts of the analytical blank obtained just before the sample measurement. Selenium concentrations in the reference materials were calculated

TABLE 2. *Mass scanning condition of the MIP-MS instrument*

Mass number	$m/z=78, 80$
Number of scanning times	3,000
Dwell times	1 ms
Integration times	3

according to the following equation (Adams et al. 1988).

$$C = w_n M_s (A_s - R B_s) / w_s W (R B_n - A_n)$$

where C is the concentration of selenium in the sample (ng/g), R is the ratio of $^{80}\text{Se}/^{78}\text{Se}$, w_n is the atomic weight of natural selenium (78.96), w_s is atomic weight of spike selenium (77.93), M_s is the amount of added spike selenium (ng), A_s is the abundance of ^{80}Se in the spike (0.01), B_s is abundance of ^{78}Se in the spike (0.9858), A_n is abundance of ^{80}Se in natural selenium (0.4961), B_n is abundance of ^{78}Se in natural selenium (0.2378), and W is the weight of the sample that is digested (g).

RESULTS AND DISCUSSION

Effect of matrix elements

Fig. 2 shows the effect of the matrix elements concentrations on ion counts of ^{80}Se . The matrix elements tested were sodium, potassium, calcium and phosphorus. The ion counts of ^{80}Se were suppressed by increasing concentrations of sodium, potassium and calcium in a similar way, but were not decreased by increasing concentration of phosphorus.

During measurements of samples containing sodium, potassium, and calcium, a whitish salt buildup was observed on the surface of sampling cone in vicinity to the orifice (0.8 mm: platinum) (Douglas and Kerr 1988). After measurement of these samples, the electrical resistance in the vicinity of the orifice of the sampling cone (which on a fresh surface is less than 0.1 ohm) greatly increased to 10^4 ohm.

The effects of sodium, potassium, calcium and phosphorus concentration on the values of $^{80}\text{Se}/^{78}\text{Se}$ and their relative standard deviations (RSDs; derived from 5 measurement for each sample) are shown in Figs. 3, 4, 5 and 6, respectively. The RSDs of the $^{80}\text{Se}/^{78}\text{Se}$ measurements show about 1% deterioration due to increasing concentrations of sodium, potassium and calcium. However, this was

Fig. 2. Relative ion count at $m/z=80$ as a function of matrix concentration. The ion count from standard solution (100 mg/liter) without matrix element is designated as 1.0. Analytical conditions are as shown in Table 2.
○—○, Ca; ●—●, P; △—△, Na; ▲—▲, K.

Fig. 3. $^{80}\text{Se}/^{78}\text{Se}$ as a function of sodium concentration. Vertical axes denote $^{80}\text{Se}/^{78}\text{Se}$ (left-hand) and RSD (%) (right-hand) relative to that in standard solution (100 mg/liter) without sodium.
 ○—○, $^{80}\text{Se}/^{78}\text{Se}$; ●—●, RSD(%)

Fig. 4. $^{80}\text{Se}/^{78}\text{Se}$ as function of potassium concentration. Vertical axes denote $^{80}\text{Se}/^{78}\text{Se}$ (left-hand) and RSD (right-hand) relative to that in standard solution (100 mg/liter) without potassium. For symbols, see Fig. 3.

Fig. 5. $^{80}\text{Se}/^{78}\text{Se}$ as a function of calcium concentration. Vertical axes denote $^{80}\text{Se}/^{78}\text{Se}$ (left-hand) and RSD (%) (right-hand) relative to that in standard solution (100 mg/liter) without calcium. For symbols, see Fig. 3.

Fig. 6. $^{80}\text{Se}/^{78}\text{Se}$ as a function of phosphorus concentration. Vertical axes denote $^{80}\text{Se}/^{78}\text{Se}$ (left-hand) and RSD (%) (right-hand) relative to that in standard solution (100 mg/liter) without phosphorus. For symbols, see Fig. 3.

not the case for phosphorus, as the ion counts of ^{80}Se were not influenced by increasing phosphorus concentrations as shown in Fig. 2. Therefore, signal suppression during measurement of these high concentration samples might be caused by the increasing electric potential of the sampling cone resulting from a build-up of ion charge on the substances deposited on the surface.

Spectroscopic interference

Spectroscopic interferences at $m/z=78$ and 80 are expected when the sample contains calcium (^{40}Ca) at high concentration. Fig. 7 shows the background mass spectrum over the mass range of $m/z=73-83$ of the Milli-Q water.

No peak was observed at mass numbers $m/z=78$ or 80 . Fig. 8 shows a mass spectrum over the mass range of $m/z=73-83$ of the sample contained 100 mg/liter calcium. Small peaks at $m/z=79$ and 81 , which can be assigned to ^{79}Br (abundance=50.69%) and ^{81}Br (49.31%), were observed but no peaks at $m/z=78$ and 80 were observed. In order to investigate calcium interference in detail, the

Fig. 7. Background mass spectrum over the mass range $m/z=73\sim 83$ when the distilled water was introduced.

Fig. 8. Background mass spectrum over mass range $m/z=73\sim 83$ when 100 mg/liter calcium standard solution was introduced. The peaks at $m/z=79$ and 81 can be assigned to ^{79}Br and ^{81}Br . No detectable peak was observed at $m/z=79$ and 80.

Fig. 9. Ion count at mass number $m/z=78$ (●) and 80 (○) as a function of calcium concentration up to 200 mg/liter.

dependency of the ion counts at $m/z=78$ and 80 on calcium concentration were measured and the result is shown in Fig 9. Signal ion counts of ^{78}Se and ^{80}Se remained constant at approximately 60 cps independently with calcium concentrations up to 200 mg/liter. As a result, calcium interferences to ^{78}Se and ^{80}Se can be ignored in the case of biological samples.

Analysis of reference materials

Selenium concentrations of the marine reference materials were determined by the isotope dilution method. ^{78}Se was spiked and the $^{80}\text{Se}/^{78}\text{Se}$ ratios were measured. Instrumental mass discrimination was corrected according to the IUPAC value ($^{80}\text{Se}/^{78}\text{Se}=2.086$) (IUPAC 1991a, b). Good agreement between the certified and found values for the marine reference samples were obtained (Table 3). Spectroscopic interferences from sulfur (SO_3 ; Yoshinaga et al. 1995)

TABLE 3. *Selenium concentrations in biological reference materials determined by isotope dilution MIP-MS. (Values are in $\mu\text{g/g}$)*

Reference material		Found	Certified
NRCC	TORT-1	6.66 ± 0.21	6.88 ± 0.47
NRCC	DORT-1	7.65	7.34 ± 0.42
NRCC	DORM-1	1.67	1.62 ± 0.12
IAEF	Fish	1.48 ± 0.01	1.35–1.70
NIES	Mussel	1.64	(1.5)*

*Reference value

and calcium, which are contained in clinical samples and marine organisms in high concentrations, were determined. Neither element had a detectable influence on $^{80}\text{Se}/^{78}\text{Se}$.

CONCLUSION

The applicability of nitrogen MIP-MS to the isotope dilution analysis of selenium was studied. No detectable interference on the isotope ratio $^{80}\text{Se}/^{78}\text{Se}$ was observed in the presence of the matrix elements sodium (up to 200 mg/liter), potassium (up to 500 mg/liter), calcium (up to 200 mg/liter) and phosphorus (up to 1,000 mg/liter).

Good agreements were obtained between the measured values of the reference materials tested and their certified values. In addition, a previous report (Yoshinaga et al. 1995) showed that sulfur (SO_3) did not detectably interfere with the isotope ratio $^{80}\text{Se}/^{78}\text{Se}$.

The preceding results show that an isotope dilution analysis of selenium with nitrogen MIP-MS can be applicable to various kinds of biological materials including clinical samples and marine organisms.

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