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Selenium isotope ratios as indicators of selenium sources and oxyanion reduction

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Abstract—Selenium stable isotope ratio measurements should serve as indicators of sources and biogeochemical transformations of Se. We report measurements of Se isotope fractionation during selenate reduction, selenite sorption, oxidation of reduced Se in soils, and Se volatilization by algae and soil samples. These results, combined with previous work with Se isotopes, indicate that reduction of soluble oxyanions is the dominant cause of Se isotope fractionation. Accordingly, Se isotope ratios should be useful as indicators of oxyanion reduction, which can transform mobile species to forms that are less mobile and less bioavailable. Additional investigations of Se isotope fractionation are needed to confirm this preliminary assessment.

We have developed a new method for measurement of natural Se isotope ratio variation which requires less than 500 ng Se per analysis and yields ±0.2‰ precision on $^{80}Se/^{76}Se$. A double isotope spike technique corrects for isotopic fractionation during sample preparation and mass spectrometry. The small minimum sample size is important, as Se concentrations are often below 1 ppm in solids and 1 μg/L in fluids. The Se purification process is rapid and compatible with various sample matrices, including acidic rock or sediment digests. *Copyright © 1999 Elsevier Science Ltd*

1. INTRODUCTION

Selenium is an essential nutrient at low concentrations and a toxin at higher concentrations (e.g., Cooper and Glover, 1974; Ganther, 1974; Skorupa, 1998). Chemically similar to sulfur, Se can be found in +6, +4, 0, and −2 valences, and in a variety of organic compounds, in natural settings (e.g., Elrashidi et al., 1987; McNeal and Balistrieri, 1989). The Se^{6+} and Se^{4+} valence states form the oxyanions selenate and selenite/biselenite, respectively. The Se oxyanions are highly soluble, but Se^{4+} adsorbs to solids more strongly (Neal and Sposito, 1989). Se^0 readily precipitates as elemental Se, and more reduced forms of Se readily form precipitates such as ferroselite, $FeSe_2$, or clausthalite, PbSe, or may be incorporated into proteins and other organic molecules. Because of differences in chemical behavior between the different oxidation states, the mobility and bioavailability of Se are strongly dependent on redox transformations. Documenting the occurrence of reduction or oxidation in nature is thus a key goal in studies of Se biogeochemistry.

Selenium redox transformations, especially reduction of selenate, are generally sluggish at environmental temperatures, and in Se-contaminated soils and sediments, selenate, selenite, elemental Se, and organically bound Se often coexist (e.g., Tokunaga et al., 1991; Zawislanski and McGrath, 1998; Zhang and Moore, 1996). Certain bacteria can respire with selenate or selenite as a terminal electron acceptor (e.g., Blum et al., 1998; Dungan and Frankenberger, 1998; Oremland et al., 1989), or oxidize elemental Se (Dowdle and Oremland, 1998), and it is likely that most of the important redox transformations are microbially mediated. Algae and other organisms are known to bioconcentrate Se and/or emit significant masses of Se as volatile alkylselenides (Fan and Higashi, 1998; Frankenberger and Karlson, 1994). The geochemistry of Se is thus strongly influenced by biology, and consideration of a variety of processes is necessary in any natural system.

Shales rich in organic matter are often rich in selenium, and Se-rich soils may develop on seleniferous shales in arid climates (e.g., Presser, 1994a; 1994b; Seiler, 1998). Irrigation practices designed to flush salts from the soils can produce Se-rich effluent. In the San Joaquin Valley of California, disposal of this water in evaporation ponds led to deformities and deaths of waterfowl at Kesterson reservoir (Ohlendorf and Santolo, 1994; Presser, 1994b), and the wastewater disposal problem has not yet been resolved. Many other areas in the western U.S. have similar problems (Seiler, 1998).

Se stable isotope ratios should be useful as indicators of biogeochemical processes and environmental sources, as are nitrogen and sulfur isotope ratios. Nitrogen isotope ratios indicate nitrogen sources (Kendall et al., 1995; Komor and Anderson, 1993) and are used to detect reduction of nitrate in groundwater, because this process fractionates the isotopes (Boettcher et al., 1990; Kohl and Shearer, 1978). Sulfur isotope ratios are used as indicators of sulfur sources and sulfate reduction (e.g., Dowuona et al., 1993; Kaplan et al., 1963; Strebel et al., 1990). Se isotope fractionation appears to be similar to sulfur isotope fractionation, according to previous research discussed below. Accordingly, isotopic shifts observed in nature should be useful as evidence of reduction processes. This is particularly valuable with Se because reduction of soluble oxyanions to insoluble Se^0 decreases the mobility and bioavailability of Se (e.g., Elrashidi et al., 1987; McNeal and Balistrieri, 1989; Tokunaga et al., 1994).

Selenium concentrations in most natural waters and soils are below 1 μg/L and 1 μg/g, respectively, and a mass spectrometry technique requiring less than 1 μg of Se per analysis is thus highly desirable. Gas-source mass spectrometry methods used

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Table 1. Compositions of natural Se and the double spike (at. %).

	^{74}Se	^{76}Se	^{77}Se	^{78}Se	^{80}Se	^{82}Se
Natural Se[a]	0.889	9.366	7.635	23.772	49.607	8.731
82/74 Spike	31.757	1.9260	0.9159	2.2908	4.0870	59.023

[a] From Wachsmann and Heumann (1992).

in previous Se isotope work (Krouse and Thode, 1962) could not attain this goal; the technique required micromole quantities of Se and involved production of SeF_6. Measurements on natural samples were limited to selenium-rich minerals and plants, and soils with unusually high concentrations. With recent developments in thermal ionization mass spectrometry (TIMS), measurements can be carried out on 500 ng Se or less (Wachsmann and Heumann, 1992). However, the precision attained with the published method is ±1 to 3‰ ($^{80}Se/^{76}Se$), and better precision is needed to apply Se isotope measurements in most natural settings.

In this paper, we describe a method for higher precision Se isotope ratio measurements (±0.2‰ on $^{80}Se/^{76}Se$) using less than 500 ng Se. We present data from new experiments and review all work to date on Se isotope fractionation by various biogeochemical processes. Previous work has demonstrated that reduction of selenate and selenite induces significant isotopic fractionation (Krouse and Thode, 1962; Rashid and Krouse, 1985; Rashid et al., 1978; Rees and Thode, 1966; Webster, 1972). Investigations of other Se transformations are needed to provide a more complete assessment of Se isotope systematics. New experimental data presented here provide initial measurements of isotopic fractionation induced by biological volatilization of Se, oxidation of elemental Se in soils, and adsorption of selenite by hydrous ferric oxides. Also, a new measurement for isotope fractionation during selenate reduction, using a less complex experiment than the previous one (Rees and Thode, 1966), is reported. Using all the Se isotopic data available to date, we discuss Se isotope systematics and potential applications.

2. ANALYTICAL METHODS

2.1. Double Isotope Spike

A double isotope spike is employed to correct for isotopic discrimination in mass spectrometry and sample preparation. At the temperatures required for thermal ionization, Se is volatile and strong fractionation of isotopes occurs, introducing a measurement bias that varies between samples and with time as data are collected on a single sample. Purification of Se for mass spectrometry may also cause isotopic fractionation. These discrimination effects can be measured and corrected for by adding two stable spike isotopes to the sample in known proportions (Eugster et al., 1969; Russell et al., 1978). Given the isotopic composition of Se (Table 1), the optimal spike isotopes are ^{74}Se and ^{82}Se. This choice of spike isotopes maximizes sensitivity to instrumental discrimination and enables simultaneous determination of $^{80}Se/^{76}Se$, $^{80}Se/^{77}Se$, and $^{80}Se/^{78}Se$ ratios.

The isotopic composition of the double spike is given in Table 1. The double spike $^{82}Se/^{74}Se$ ratio, measured by mass spectrometry, was checked via isotope dilution of the ^{82}Se and ^{74}Se solutions against a standard solution. The two results agree within the ±1% uncertainty of the isotope dilution determination (gravimetric calibration was not attempted because the ^{74}Se spike is costly, and the small amount purchased could not be precisely weighed). Because of the uncertainty in the double spike composition, the calculated isotope ratios may be systematically shifted by up to 1.2‰/a.m.u. difference. However, results are reported as per mil deviations from standard ratios, as are done with all light stable isotope ratios. The uncertainty in the double spike $^{82}Se/^{74}Se$ ratio does not significantly affect these relative results.

2.2. Mass Spectrometry

A Finnigan MAT 261 mass spectrometer operating in negative ion mode was used for this study. 100 to 500 ng Se (as selenious acid) is loaded onto single rhenium filaments. Barium hydroxide is added to the filament to enhance ionization (Wachsmann and Heumann, 1992); 1 μL of a saturated barium hydroxide solution (24 μg Ba) is placed on the filament and dried. The volatility of Se (SeO_2 and Se^0 vaporize at 350° and 610°C, respectively) is reduced by adding graphite to the sample. The sample and 0.2 μg colloidal graphite are taken up in de-ionized water, placed on top of the dried $Ba(OH)_2$, and gently evaporated to dryness. This technique is easier to perform than the previous double filament technique (Wachsmann and Heumann, 1992) and produced stronger ion beams in our instrument. They reported ion beams of greater than 10^{-11} A $^{80}Se^-$ on 0.5 μg loaded Se with their technique, but we were unable to obtain more than 3×10^{-12} A $^{80}Se^-$. The loading technique reported here yielded ion beams of approximately 10^{-11} A $^{80}Se^-$.

Filaments are heated to 900°C over a 5-min interval. At 900°C, Se^- ions are observed, along with rapidly diminishing Br^- beams at masses 79 and 81 arising from traces of Br in the samples and reagents. Maximum signal intensity for Se^- occurs between 950°C and 1000°C, although data are usually collected at lower temperature to minimize drift in instrumental discrimination. Measurements of $^{82}Se/^{80}Se$, $^{78}Se/^{80}Se$, $^{77}Se/^{80}Se$, $^{76}Se/^{80}Se$, and $^{74}Se/^{76}Se$ ratios are made in a sequence dictated by the configuration of the mass spectrometer. It is also helpful to monitor the PO_3^- beam (major mass at 79 a.m.u., minor masses at 80 and above), which may be very large for phosphate-contaminated samples. If a large signal is observed, comparison with the 81 a.m.u. beam is necessary to distinguish PO_3^- from Br^-. The mass spectrometer is equipped with 5 Faraday collectors, and the two ion beams for each ratio are measured simultaneously to eliminate errors caused by temporal variations in intensity. The detectors cannot be positioned far enough apart to measure the $^{80}Se/^{74}Se$ ratio, and each ratio requires a unique detector spacing that usually cannot be used to measure other ratios. Accordingly, the ratios are measured in a series of steps given in Table 2. Use of this sequence minimizes the effects of drift in instrumental discrimination and amplifier gain on the $^{80}Se/^{76}Se$ determinations.

2.3. Data Reduction

An iterative data reduction scheme similar to that used in previous applications of the double spike technique (Eugster et al., 1969; Russell

Table 2. Optimal measurement sequence.

Step number	Collector number				
	1	2	3	4	5
1			^{80}Se		^{82}Se
2		^{78}Se		^{80}Se	
3	^{76}Se	^{77}Se			^{80}Se
4	^{74}Se		^{76}Se		

et al., 1978; Skulan et al., 1997), is used to extract the sample's $^{80}Se/^{76}Se$, $^{80}Se/^{77}Se$, and $^{80}Se/^{78}Se$ ratios from the measurements. The calculations follow a nested iteration scheme whereby the instrumental discrimination and natural isotope composition are successively refined:

1. A trial value for the $^{82}Se/^{74}Se$ ratio of the double spike, with the natural Se removed, is calculated:

$$\left(\frac{^{74}Se}{^{82}Se}\right)_{s,\,\mathrm{trial}} = \frac{\left(\frac{^{74}Se}{^{80}Se}\right)_m - \left(\frac{^{74}Se}{^{80}Se}\right)_n}{\left(\frac{^{82}Se}{^{80}Se}\right)_m - \left(\frac{^{82}Se}{^{80}Se}\right)_n} \times \frac{\left[1 - \left(\frac{^{82}Se}{^{80}Se}\right)_m \Big/ \left(\frac{^{82}Se}{^{80}Se}\right)_s\right]}{\left[1 - \left(\frac{^{74}Se}{^{80}Se}\right)_m \Big/ \left(\frac{^{74}Se}{^{80}Se}\right)_s\right]}, \quad (1)$$

where the subscripts m, n, and s refer to the measured mixture, the assumed natural Se composition, and the assumed double spike composition, respectively. The $^{74}Se/^{80}Se$ ratios are obtained by multiplying $^{74}Se/^{76}Se$ by $^{76}Se/^{80}Se$. The reader will note that this equation takes into account the significant ^{80}Se and ^{76}Se impurities in the double spike.

2. The trial $^{82}Se/^{74}Se$ ratio of the double spike is compared to the assumed ratio to calculate the discrimination. An exponential discrimination law is used (see explanation below); the measured ratio r is related to the unfractionated ratio r_0 by

$$r = r_0 \left(\frac{m_1}{m_2}\right)^p, \quad (2)$$

where m_1 and m_2 are the masses of the two isotopes and p is the parameter that determines the degree of fractionation.

3. Using the calculated value for p, the measured ratios are corrected.
4. Step 1 above is performed using the corrected ratios; steps 1 through 3 are repeated iteratively until the values converge.
5. A trial value for the sample's $^{80}Se/^{76}Se$, $^{80}Se/^{77}Se$, or $^{80}Se/^{78}Se$ ratio, with the double spike subtracted, is calculated. Using this result (2) and the assumed natural composition, trial values for the sample's $^{74}Se/^{80}Se$ and $^{82}Se/^{80}Se$ are calculated.
6. A new series of iterations that refine the instrumental discrimination are then performed using the sample's revised isotopic composition.
7. Several series of iterations are performed until the sample's isotopic composition converges.

The exponential fractionation relation given in 2 is used to model the decrease in fractionation with increasing mass (e.g., $^{82}Se/^{80}Se$ fractionation is less than $^{76}Se/^{74}Se$ fractionation). This relation has been shown to closely approximate calcium isotope fractionation during thermal ionization (Russell et al., 1978), and it works well for selenium, according to data from several mass spectrometer runs (Fig. 1) The accuracy of the exponential model in approximating fractionation by natural processes has not been established, but this does not affect the accuracy of the present data reduction routine significantly. For example, tests with synthetic data indicate that if the true natural fractionation law were linear, the error caused by use of the exponential law in data reduction would be less than 0.08‰ over a ±40‰ range in $^{80}Se/^{76}Se$. As measurement precision improves, this issue may need to be revisited.

2.4. Se Purification

The double isotope spike should be added as early as possible in the sample preparation process, but the spike must be in the same chemical form as the sample Se to be spiked. If necessary, samples are digested to convert selenate to selenite by adding HCl to attain a 6 N HCl solution and heating for 20 min at 100°C. The selenium is then purified for mass spectrometry via hydride generation. The HCl concentration is adjusted to 4 mol/L, and an $NaBH_4$ reductant solution is added. Tetravalent Se in the solution is reduced to form H_2Se, which is scrubbed from the sample solution and trapped in concentrated nitric acid (Tanzer and Heumann, 1991). Hydride generation has been used extensively to enhance Se concentration measurement techniques, and is compatible with strongly acidic soil digests (Dedina and Tsalev, 1995). Hydride generation has also been applied as a Se purification method for isotope dilution mass spectrometry studies (Heumann and Radlein, 1989; Heumann and Wachsmann, 1989; Tanzer and Heumann, 1991). Sb, As, Bi, Ge, In, Pb, Te, Sn, and Tl may also form volatile hydrides (Dedina and Tsalev, 1995), but these do not adversely affect Se mass spectrometry. Anion exchange chromatography could also be used in some cases but is problematic for soil or rock digests because Se^{4+} forms an uncharged species under the highly acidic conditions needed to stabilize dissolved metals.

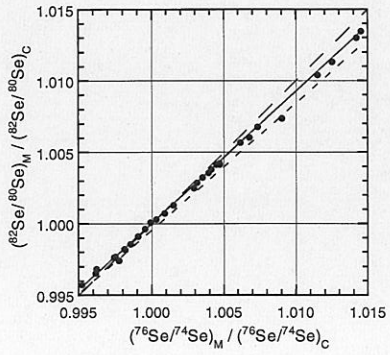

Fig. 1. Comparison of models for isotopic discrimination during thermal ionization to data from several mass spectrometer runs. Data are mean values from individual blocks of 10 measurements, normalized to the discrimination-corrected values for each spiked sample or spiked standard. Exponential, linear, and "Rayleigh" fractionation laws, are given by the solid, dashed, and short-dashed lines, respectively. The Rayleigh fractionation line represents expected results for progressive removal of Se from the filament by a process in which fractionation is proportional to the inverse square root of the mass (Russell et al., 1978). The linear fractionation line is for a process in which fractionation does not depend on mass.

The hydride generation technique alters the isotope ratios, but the double spike corrects for this effect. Because the process involves chemical reduction and the yield is typically less than 90%, significant isotopic fractionations are expected. We have observed $^{80}Se/^{76}Se$ fractionations greater than 4‰. However, the double isotope spike corrects for the induced fractionation. The double spike is added to the sample prior to Se purification; otherwise the procedure followed is that of previous studies (Tanzer and Heumann, 1991) with three modifications: (1) Ferric iron is removed via cation exchange resin whenever the Fe^{3+} concentration in the reaction vessel is greater than 100 mg/L because it can interfere with Se reduction (Dedina and Tsalev, 1995). (2) Organic compounds transferred along with the H_2Se are destroyed by adding H_2O_2 as the sample is evaporated to dryness. (3) The entire hydride generation apparatus is constructed of fluoropolymer plastics and is rinsed with 8 mol/L HNO_3 between samples.

2.5. Analytical Precision

A provisional internal standard, MH495, is analyzed each day Se analyses are performed (an interlaboratory standard has not yet been established). Table 3 gives $^{80}Se/^{76}Se$, $^{80}Se/^{77}Se$, and $^{80}Se/^{78}Se$ ratios calculated from 13 recent MH495 analyses. The standard deviation of the 13 $^{80}Se/^{76}Se$ ratios is 0.12‰ of the mean. The double spike composition and the data collection routine are designed for optimal $^{80}Se/^{76}Se$ precision, and thus the $^{80}Se/^{78}Se$ variation is slightly greater. The $^{80}Se/^{77}Se$ precision is presently not as good as that of $^{80}Se/^{76}Se$ and $^{80}Se/^{78}Se$ because of an unidentified isobaric interference at 77 a.m.u. The double spike routine successfully corrects for large variations in instrumental mass discrimination; uncorrected $^{80}Se/^{76}Se$ ratios drifted an average of 4‰ and a maximum of 12‰ during these analyses.

Expanded notation is necessary for unambiguous reporting of selenium isotope data. Measurements can be reported as $\delta^{80/76}Se$, $\delta^{80/77}Se$, and $\delta^{80/78}Se$, the per mil deviations of the indicated ratios from those

Table 3. Measured isotope ratios of the MH495 standard.

Analysis	$^{80}Se/^{76}Se$	$^{80}Se/^{77}Se$	$^{80}Se/^{78}Se$	ΔD (‰)[a]
Unprocessed standards:				
1/7/98-1	5.3262	6.5163	2.0923	12.0
1/7/98-2	5.3261	6.5220	2.0925	1.5
1/9/98	5.3265	6.5242	2.0927	5.2
5/28/98	5.3252	6.5194	2.0922	3.2
5/29/98	5.3249	6.5243	2.0927	11.6
6/2/98	5.3258	6.5245	2.0927	4.4
6/3/98-1	5.3253	6.5293	2.0930	1.9
6/3/98-2	5.3244	6.5241	2.0927	5.2
6/4/98	5.3255	6.5027	2.0923	0.9
Standards processed through hydride generation:				
1/8/98-proc	5.3261	6.5402	2.0929	1.2
1/9/98-proc	5.3263	6.5282	2.0930	2.8
5/21/98-proc	5.3265	6.5270	2.0928	1.4
5/22/98-proc	5.3257	6.5285	2.0930	1.7
Mean	5.3257	6.5239	2.0927	
Std. dev.	±0.0006	±0.0082	±0.0003	
‰ Std. dev.	±0.12‰	±1.31‰	±0.13‰	

[a] Change in the instrumental discrimination over the course of analysis, expressed as change in $^{80}Se/^{76}Se$.

of a standard. The $^{82}Se/^{76}Se$ ratios measured using gas source mass spectrometry can be reported as $\delta^{82/76}Se$. Results reported in this paper are relative to our MH495 standard; an interlaboratory Se isotope standard has not been established. Although the Canyon Diablo Troilite standard was used in the past (Krouse and Thode, 1962), we chose a synthetic standard because of the scarcity and potential heterogeneity of the Canyon Diablo Troilite material.

Results from duplicate analyses on various sample types are given in Table 4. In each case, duplicate aliquots of solution (sediment or soil digest, microbial suspension medium, or dissolved microbial Se^0 precipitate) were double spiked, processed, and analyzed. Duplicates were processed and analyzed from 1 to 2 months after the original samples, so that long-term reproducibility could be established. Based on the reproducibility of these samples and the repeated analysis of the MH495 standard (Table 2), we estimate our present precision at ±0.2‰ (95% confidence). This is similar to precision attained in a recent study of Ca isotope ratios using the double spike technique (Skulan et al., 1997).

In Fig. 2, $\delta^{80/77}Se$ and $\delta^{80/78}Se$ are plotted against $\delta^{80/76}Se$ for a suite of samples. The $\delta^{80/78}Se$ data are fully consistent with the $\delta^{80/76}Se$ data and an exponential fractionation law, and in general can be used to confirm results. However, $\delta^{80/78}Se$ results are somewhat less useful because the variations in $\delta^{80/78}Se$ are smaller. The $\delta^{80/77}Se$ data in Fig. 2 exhibit considerable deviation from the expected $\delta^{80/77}Se$ vs. $\delta^{80/76}Se$ relationship, and we attribute this to the same interfering species that caused the lack of precision in the standard analyses.

Because the isotope ratios are not all measured at the same time, errors are introduced by changes in discrimination between the four steps of the measurement cycle. However, use of the measurement sequence given in Table 2 eliminates most of this error when discrimination changes linearly with time. Thus a steady filament temperature should be maintained during data collection to minimize nonlinear discrimination drift, and precision is degraded if changes in instrumental discrimination are highly nonlinear with time (e.g., after abrupt changes in filament temperature).

The mass of Se recovered in blank solutions processed through the hydride generation procedure is approximately 0.2% of the mass of the previously processed sample. The Se blank did not vary in response to changes in the amounts of HCl or $NaBH_4$ solution used. Sample carryover appears to be the source of the blank, in the form of a recalcitrant Se^0 precipitate that survives the washing step. This level of cross contamination is insignificant if successive samples contain roughly the same mass of Se; sample aliquot sizes are chosen to ensure this is the case. When the carryover must be eliminated, overnight soaking of the entire apparatus in concentrated HNO_3 reduces the carryover by at least a factor of 10. Se contamination on filaments loaded with $Ba(OH_2)$ and graphite, or $Ba(OH_2)$ alone, was minimal, and produced Se ion beams 10^4 smaller than the smallest sample beams.

3. EXPERIMENTAL METHODS

Se isotope fractionation caused by selenate reduction to selenite has been studied previously (Rees and Thode, 1966), but the experiments performed were complex and involved separation of the effects of two reduction steps. We designed a simpler experiment involving separation of Se^{4+} from Se^{6+} by coprecipitation of Se^{4+} on hydrous ferric oxides. Several parallel experiments containing 20 mL of 4 N HCl with 100 mg/L Se as selenate were heated at 70°C for varying amounts of

Table 4. Results of duplicate Se purification and analysis.

Sample name, Matrix type	Original $\delta^{80/76}Se$	Uncertainty $(2s)$[a]	Duplicate $\delta^{80/76}Se$	Uncertainty $(2s)$[a]	Difference (‰)
T2S1-C, Sediment TAD[b]	2.53‰	±0.18‰	2.64‰	±0.12‰	0.11‰
T2S11-D, Sediment TAD[b]	2.21‰	±0.06‰	2.07‰	±0.15‰	0.14‰
WC-t4-15-VI-Se^0, Microbial precipitate	−5.82‰	±0.04‰	−5.83‰	±0.04‰	0.01‰
WC-t4-30-VI-Se4+, Microbial medium	9.45‰	±0.03‰	9.34‰	±0.02‰	0.11‰
P11A, Soil TAD[b]	5.36‰	±0.08‰	5.67‰	±0.07‰	0.31‰
Se(IV) stock sol'n	2.56‰	±0.03‰	2.68‰	±0.06‰	0.12‰

[a] Standard error based on variation between blocks of data from one filament ($3 < n < 6$).
[b] TAD: total acid digest of sediment or soil.

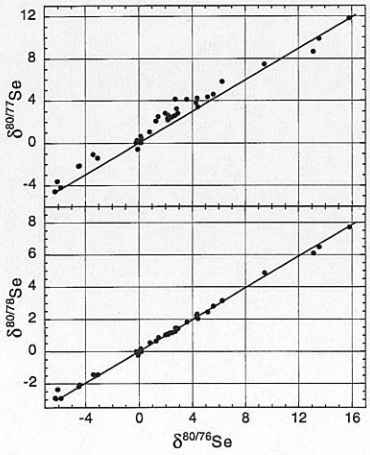

Fig. 2. Correlation of $\delta^{80/77}$Se and $\delta^{80/78}$Se with $\delta^{80/76}$Se for a suite of standard and sample runs. 95% confidence limits are approximately the size of the data points. The theoretical relationships between the ratios, based on an exponential fractionation law, are given by solid lines and are essentially indistinguishable from those based on a linear fractionation.

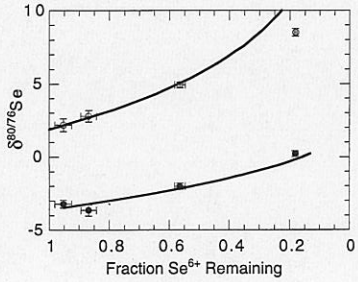

Fig. 3. Isotopic fractionation accompanying reduction of Se^{6+} to Se^{4+} in hot HCl. The fraction of Se^{6+} remaining is given by f. Closed circles give $\delta^{80/76}$Se values for the produced Se^{4+}; open circles give values for the remaining Se^{6+}. The lines represent a constant fractionation model with $\varepsilon = -5.5‰$.

time up to 300 min to achieve up to 82% reduction. Reduction was stopped by adding 25 mL cold water to cool and dilute the HCl. The solutions were then cooled further and stored at 4°C overnight. Selenate and selenite concentrations were determined by hydride-generation atomic absorption spectrometry using standard techniques. Selenate and selenite were separated by coprecipitation of the selenite onto ferric hydroxide (Chau and Riley, 1965). A 10 mL aliquot of the solution was diluted to 50 mL, 5 μg Fe^{3+} was added, and the pH was adjusted to 4.0 to precipitate ferric hydroxide. The precipitate was recovered by filtration and dissolved in 0.2 N HNO_3. Fe was removed by passing the solution through cation exchange resin. Aliquots of this solution and of the selenate-bearing filtrate were then purified for isotopic analysis.

Se isotope fractionation caused by selenite adsorption onto ferric hydroxide was also measured. Amorphous ferric hydroxide was prepared by creating a solution containing 10 μg/L Fe^{3+} at a pH of 1.5, then adjusting the pH to 4.0. After the precipitation was complete, an Na_2SeO_3 solution was added to attain 2 mg/L Se. The ferric hydroxide was kept suspended by stirring for 30 min, after which 82% of the selenite was adsorbed. The precipitate was collected onto a 0.45 μm pore size filter and was dissolved in 0.2 N HNO_3. Fe was removed by passage of the solution through a cation exchange resin column. Aliquots of the sorbed and dissolved Se fractions were then processed for isotopic analysis.

Isotopic fractionation caused by oxidation of reduced Se in soil was measured using archived samples from an incubation study. That study (Zawislanski and Zavarin, 1996) measured rates of Se oxidation; experimental details can be found therein. Briefly, soil with an initial total Se concentration of 93 mg/kg Se, of which approximately 72% was Se^0 and approximately 17% was organically bound, was incubated for 400 d in air at 35°C and 21% volumetric moisture content. Approximately 42% of the Se^0, and 18% of the organically bound Se, were oxidized to soluble forms; this fraction was extracted with water and analyzed. In the present study, Se isotope ratios were determined for archived water extracts (soluble Se), NaOH extracts (organically bound Se), and total acid digests (total Se).

Samples of volatile Se generated by soil incubations in experiments by Zhang and Frankenberger were obtained. Details of the incubation technique can be found in a recent publication (Zhang and Frankenberger, 1999). Se-contaminated soils from the San Luis Drain and an evaporation pond at Kesterson wildlife refuge were incubated for 2 d at 20% moisture content. Each soil was incubated with and without an organic amendment (an acid-hydrolyzed casein) which accelerated volatilization. Volatilized Se was recovered in alkali–peroxide traps. Se isotope ratios were measured in these solutions and compared to the Se isotope ratios of total soil Se recovered from splits of the starting soil by digestion in a concentrated nitric acid–H_2O_2 solution.

Samples of volatile alkylselenides generated by algal cultures in a recent study (Fan et al., 1998) were obtained and analyzed, along with the stock selenite solution used as a Se source in the growth medium. Algal culture details can be found in the original paper (Fan et al., 1998) and are summarized briefly here. A cyanophyte-dominated algal mat cultured from a Se-laden drainage pond was isolated and grown in an $f/2$ seawater medium in the laboratory. The culture was continuously aerated, and volatilized Se in the outflow was collected in alkali–peroxide traps. Before processing for mass spectrometry, Se in these traps was converted to selenite form by first oxidizing all of the Se to selenate by heating with H_2O_2, then reducing the selenate to selenite by digestion in HCl.

4. EXPERIMENTAL RESULTS

In the selenate reduction experiment, the produced selenite was enriched in the lighter isotope as expected. The results are plotted in Fig. 3. A model assuming a constant kinetic fractionation for selenate reduction fits the data well except for the last time point. The least-squares fit gives an instantaneous fractionation of $\varepsilon = -5.5‰$ for the selenate reduction reaction. This model assumes negligible back reaction of selenite to selenate. The rate of the backward reaction must be small because this digestion is known to convert greater than 99% of the selenate to selenite (Brimmer et al., 1987). However, toward the end of the experiment, as the selenate concentration becomes small relative to the selenite concentration, the cumulative effects of small amounts of back reaction are more likely to be important. This appears to be the case with this experiment.

The adsorption experiment showed little isotopic fractionation. The $\delta^{80/78}$Se value of the dissolved selenite was 2.73‰ ± 0.14‰ ($2s$), whereas the selenite adsorbed onto the ferric hydroxide yielded a value of 2.20‰ ± 0.40‰. These data suggest a slight enrichment of the lighter isotopes in the sorbed phase, but this effect is barely distinguishable at the 95% confidence level. This result is similar to results reported for sulfate adsorption onto goethite (Van Stempvoort et al., 1990), which found the sorbed fraction enriched in the lighter isotope by 0.3‰.

In the soil oxidation experiment, isotopic fractionation was small or zero. Data are presented in Table 5. The $\delta^{80/76}$Se value of evolved soluble Se is close to those of the starting Se^0 and organically bound Se from which it oxidized. Elemental Se was the source of approximately 95% of the evolved soluble Se.

Table 5. Results from the soil–Se oxidation experiment.

Se fraction	Fraction of total Se	$\delta^{80/76}$Se (‰)
Before incubation:		
Total Se		3.01 ± 0.15
Soluble Se	4.6%	4.17 ± 0.15
Organic Se	17%	3.20 ± 0.15
Elemental Se	72%	2.89 ± 0.40[a]
After incubation:		
Soluble Se	43%	2.88 ± 0.15
Soluble Se evolved	38%[a]	2.74 ± 0.19[a]
Organic Se	15%	2.71 ± 0.15

[a] Calculated by mass balance.

The $\delta^{80/76}$Se of the starting Se^0 was calculated from mass balance because extracts of this component alone were not available. This value has a greater uncertainty because extracts of the sorbed fraction and the carbonate fraction (6% of total) were not available for analysis and their contribution to the mass balance could not be included. Nonetheless, these results indicate that isotopic fractionation during reoxidation of Se previously added to soil by reduction is less than 0.5‰.

Results from the algal volatilization experiments indicate that Se isotope fractionation caused by uptake and volatilization by algae is minor. The $\delta^{80/76}$Se value of the selenite added to the growth medium was 2.5‰ ± 0.15‰. The results for the volatilized Se were 2.57‰ ± 0.15‰ and 1.42‰ ± 0.15‰ for early and late time periods in the experiment, respectively. The earlier sample was taken as the algae were growing rapidly, whereas the later sample was taken after growth had slowed. An isotopic fractionation of approximately one per mil is suggested by the latter. It is impossible to separate potential fractionation effects caused by uptake and volatilization, but the results suggest that both processes induce little fractionation. For example, a scenario in which both uptake and volatilization cause strong enrichment in the lighter isotopes can be ruled out.

Se volatilized by the incubated soils had $\delta^{80/76}$Se values within 0.6‰ of the total soil Se values. Results are given in Table 6. These results suggest that Se volatilization under these conditions induces little or no fractionation. However, it is possible that the $\delta^{80/76}$Se values of the Se species that were consumed to produce the volatile Se differed greatly from the total soil values and that the observed volatile Se values resulted from large isotopic fractionations. The likelihood that this process would result in values close to the total soil values in both experiments is small.

Table 6. Results of the soil–Se volatilization experiment.

Sample	$\delta^{80/76}$Se (‰)
Total Se Digests:	
Kesterson	3.76 ± 0.15
San Luis Drain	2.41 ± 0.15
Volatilized Se:	
Kesterson-unamended	3.26 ± 0.15
Kesterson-amended	3.20 ± 0.15
San Luis Drain-unamended	2.90 ± 0.15
San Luis Drain-amended	2.17 ± 0.15

5. DISCUSSION

5.1. Se Isotope Systematics

Previous Se isotope studies, combined with the results reported here, provide sufficient data for a preliminary assessment of Se isotope systematics and potential applications. Se isotopic fractionations accompanying various biogeochemical transformations are summarized in Table 7. The most important conclusion is that the dominant isotope fractionating processes are selenate and selenite reduction. Thus, if Se isotope fractionation is observed in nature, it provides strong evidence that reduction is occurring. Kinetic isotope effects have already been exploited as indicators of sulfate and nitrate reduction in groundwater (Boettcher et al., 1990; McMahon and Bohlke, 1996; Strebel et al., 1990) and in other settings (Kaplan et al., 1963; Zaback et al., 1993). Because of the chemical similarities between S and Se (both are group VIA elements), sulfur isotope systematics can serve as a guide to predict Se isotope systematics. It is well known that sulfide produced by bacterial or inorganic reduction of sulfate is enriched in the lighter isotopes (e.g., Habicht and Canfield, 1997; Kaplan and Rittenberg, 1964). Sulfur isotope ratios have been used to unravel diagenetic processes in ocean and lake sediments (Bruechert and Pratt, 1996; Tuttle and Goldhaber, 1993; Zaback et al., 1993), sulfate reduction in groundwater (Strebel et al., 1990), and sulfate reduction in lakes (Dickman and Thode, 1990).

Table 7. Instantaneous isotopic fractionation ε caused by biogeochemical transformations.

Study	Transformation	Reacting agent	Measured ratio	ε, (‰)	ε ($\delta^{80/76}$Se)[a], (‰)
(Rees and Thode, 1966)	Se^{6+} to Se^{4+}	HCl, 25°C	$^{82}Se/^{76}Se$	−18	−12
This study	Se^{6+} to Se^{4+}	HCl, 70°C	$^{80}Se/^{76}Se$	−5.5	−5.5
(Krouse and Thode, 1962)	Se^{4+} to Se^0	NH_2OH	$^{82}Se/^{76}Se$	−15	−10
(Rees and Thode, 1966)	Se^{4+} to Se^0	Ascorbic acid	$^{82}Se/^{76}Se$	−19	−13
(Webster, 1972)	Se^{4+} to Se^0	NH_2OH	$^{80}Se/^{74}Se$	−10	−7
(Rashid and Krouse, 1985)	Se^{4+} to Se^0	NH_2OH	$^{82}Se/^{76}Se$	−11	−7
(Rashid et al., 1978)	Se^{4+} to Se^0	Microbes	$^{82}Se/^{76}Se$	−5 to −40	−3 to −27
This study	Soil–Se oxidation	Microbes?	$^{80}Se/^{76}Se$	<0.5	<0.5
This study	Se^{4+} adsorption	$Fe(OH)_3 \cdot nH_2O$	$^{80}Se/^{76}Se$	<0.5	<0.5
This study	Se volatilization	Algal culture	$^{80}Se/^{76}Se$	<1.1	<1.1
This study	Se volatilization	Soil (Microbes)	$^{80}Se/^{76}Se$	<0.6	<0.6

[a] $^{82}Se/^{76}Se$ values are converted to $^{80}Se/^{76}Se$ in this column.

The results of the Se^0 oxidation and selenite sorption experiments are consistent with similar experiments with sulfur and theoretical considerations. Simple oxidation of solid-phase Se^0 should not fractionate the isotopes because there is no opportunity for isotope selection as a solid particle is dissolved and the surface atoms are completely removed. Oxidation of elemental sulfur by *Thiobacillus thiooxidans* does not fractionate sulfur isotopes (Jones and Starkey, 1962). Adsorption of selenium oxyanions does not involve large changes in the bonding environment of the Se and thus does not strongly fractionate isotopes. Similar results have been obtained for sulfate sorption (Van Stempvoort et al., 1990). For the same reason, precipitation of SeO_3^{2-} or SeO_4^{2-}-bearing minerals should not fractionate Se isotopes significantly. Little or no sulfur isotope fractionation is observed upon precipitation of sulfate minerals from seawater (Thode and Monster, 1965). In general, Se isotope systematics are roughly similar to those of sulfur, and Se isotope applications can be expected to parallel those of sulfur isotopes to some extent.

On a more detailed level, several questions remain. First, no experiments have yet been conducted on Se isotope fractionation during selenite oxidation. More importantly, microbial metabolic pathways differ between S and Se, and selenate respiration does not occur by the same pathways as sulfate respiration (Oremland et al., 1989). Thus, general similarities between S and Se reduction processes are expected, but detailed studies of microbial reduction of selenate and selenite are needed. The only previous study (Rashid et al., 1978) was published only as an extended abstract, and investigated only selenite reduction. We are currently conducting studies of pure cultures of selenium-reducing bacteria, soil slurries, and natural systems in order to address this issue (Herbel et al., 1998). Results from bacterial sulfur isotope fractionation studies (Kaplan and Rittenberg, 1964; Kemp and Thode, 1968) can be extended to suggest that Se isotope fractionations will be variable and generally less than the abiotic fractionations. Another reaction not yet studied is bacterial disproportionation. It has been suggested recently (Habicht and Canfield, 1997) that disproportionation reactions greatly influence S isotope systematics, and similar reactions could occur with Se as well.

Se isotope fractionation during uptake of Se by higher plants has not yet been measured directly. However, field data and results from S isotope studies suggest that this process involves little or no fractionation. Plants from the San Francisco estuary system and from evaporation ponds in the San Joaquin Valley have isotope ratios close to coexisting waters and soils or sediments (Herbel, 1997; Johnson et al., submitted). Sulfur assimilated by plants and algae is not fractionated relative to that in the growth medium (Chukhrov et al., 1980; Kaplan et al., 1963). Thus, it is expected that plant-induced fractionations are small, but the definitive experiments have not yet been done. In summary, whereas presently available data suggest applications for Se isotopes similar to those of S isotopes, results from further studies are needed before these similarities can be examined in detail.

5.2. Se Isotope Applications

Se isotope ratio data may provide evidence for Se reduction in reducing wetland sediments. Wetlands are a major focus of concern over Se impacts in the environment, both because they are the most important sites of wildlife exposure (Seiler, 1998) and because artificial wetlands have been constructed to remove selenium from agricultural or industrial wastewater (Hansen et al., 1998; Terry and Zayed, 1998). An understanding of the removal process is needed to optimize the management of the wetlands. Reduction of the selenate to elemental Se in the reducing environment of the wetland sediments is a probable mechanism, but plant or algal uptake, volatilization, and sorption are also possible contributors to Se mass balance. Isotope ratio measurements could be useful in this case: If the selenate remaining in solution becomes progressively enriched in the heavier isotopes along a flow path, one would conclude reduction is occurring. Alternatively, a decrease in selenate concentration along the flow path without an isotope shift would suggest one of the other removal mechanisms is operative, as they do not fractionate the isotopes appreciably.

Se isotopes may also be useful in detecting remobilization of previously reduced Se that is sequestered in sediments or aquifer materials. If the reduced species are enriched in the lighter isotopes because of the previous reduction, oxidation and remobilization of these species may be detected through isotope ratio analyses. For example, if pore waters in a sediment are shifted toward lower $\delta^{80/76}Se$ values relative to the overlying water column, this would suggest remobilization of reduced Se in the sediments. This assumes little or no fractionation during the oxidation process; such an assumption is supported by the results of the present study.

Se isotope measurements can also be used to trace the transport of anthropogenic Se in the environment. Industrial inputs to the environment may be isotopically distinct from the natural background because of natural isotopic contrasts in the Se sources or fractionation during purification of industrial Se and/or wastewater processing. In a system with little or no reduction of Se oxyanions, isotopic tracing of anthropogenic inputs would be straightforward. In other systems, isotopic tracing may still be possible if the effects of reduction can be estimated.

6. CONCLUSIONS

The method described in this paper makes precise measurement of Se isotope ratios in environmental samples possible for the first time. The combination of double-spike-corrected TIMS analysis and purification by hydride generation enables relatively rapid analysis of less than 500 ng Se. Dissolved Se concentrations in natural waters and soils are often less than 1 μg/L and 1 μg/g, respectively. Typically, the concentrations of individual redox species are less than these values, and separate analysis of each species is desirable. With the new method, sufficient quantities of Se are present in samples of less than a few liters of water or a few grams of soil or sediment. The current $\delta^{80/76}Se$ precision of $\pm 0.2‰$ (95% confidence) is sufficient for detection of Se reduction reactions, which produce fractionations of 10‰ or less. The TIMS method offers two major advantages over gas source mass spectrometry. The hydride generation purification step is less hazardous and less time consuming than the purification and fluorination procedures required to produce SeF_6. The mass of Se required for

TIMS measurements is many times smaller than what would be required for even the most recent gas-source methods.

Se isotope fractionation experiments reported here indicate strong fractionation during selenate reduction to selenite, and little or no fractionation for selenite sorption, oxidation of reduced Se in soils, and Se volatilization by algae and soil samples. These results, combined with previous work and similarities with sulfur isotopes, indicate that selenate reduction and selenite reduction are the major isotope fractionating processes identified to date. However, additional work is needed to provide a more complete understanding of Se isotopic fractionation in natural environments. Work is in progress with bacterial reduction and with field samples to address these issues.

Se isotope systematics are broadly similar to those of sulfur, and as with sulfur, isotope measurements should provide information on sources and chemical transformations in the environment. Se oxyanion reduction is a critical transformation because it greatly influences the mobility and bioavailability of Se. It is likely that measurement of $\delta^{80/76}$Se can provide a means of detecting Se reduction. In some locations, anthropogenic Se inputs such as those from oil refining, municipal wastewater, or fly ash disposal may be distinguished from naturally occurring Se through isotopic measurements.

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