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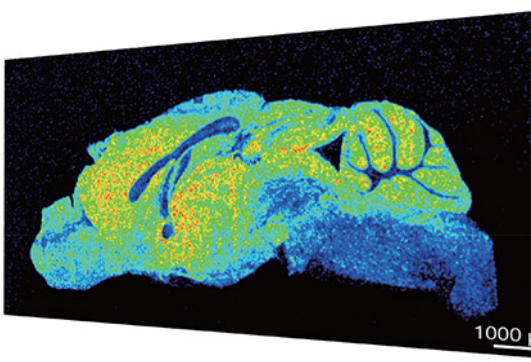

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Measurement of the $\delta^{34}S$ value in methionine by double spike multi-collector thermal ionization mass spectrometry using Carius tube digestion†

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Methionine is an essential amino acid and is the primary source of sulfur for humans. Using the double spike (^{33}S-^{36}S) multi-collector thermal ionization mass spectrometry (MC-TIMS) technique, three sample bottles of a methionine material obtained from the Institute for Reference Materials and Measurements have been measured for $\delta^{34}S$ and sulfur concentration. The mean $\delta^{34}S$ value, relative to Vienna Canyon Diablo Troilite (VCDT), determined was 10.34 ± 0.11‰ (n = 9) with the uncertainty reported as expanded uncertainties (U). These $\delta^{34}S$ measurements include a correction for blank which has been previously ignored in studies of sulfur isotopic composition. The sulfur concentrations for the three bottles range from 56 to 88 μg/g. The isotope composition and concentration results demonstrate the high accuracy and precision of the DS-MC-TIMS technique for measuring sulfur in methionine. Published in 2010 by John Wiley & Sons, Ltd.

Methionine is a sulfur-bearing amino acid that, although not synthesized in humans, is essential for human metabolism, for proper body functioning and for overall good health. As a consequence methionine must be obtained from food sources such as beans, nuts, and seeds as well as meats, fish and dairy products. Renewed focus on the sulfur-containing amino acids methionine and cysteine has been brought about not only because of their link to diseases including cardiovascular disease,[1,2] Alzheimer's disease[3,4] and diabetes,[5] but also because of their potential as a personalized biomarker for early cancer detection.[6] Typically, natural stable sulfur isotopes have been used in sulfur metabolism studies[7–11] but, more recently, enriched ^{34}S has been used as metabolic tracer, where the amino acids methionine and cysteine are labeled with ^{34}S and, after protein synthesis within the human body, the isotope composition is determined.[6] The resulting isotope composition of sulfur in proteins metabolized by tumor cells differs from that of proteins metabolized from normal, non-cancerous, cells thus providing an early cancer detection technique.

Sulfur (S) has four stable isotopes: ^{32}S, ^{33}S, ^{34}S and ^{36}S, with approximate relative isotopic abundances of 95%, 0.75%, 4.2% and 0.015%, respectively. The isotope composition variations are expressed as relative differences between a sample and a standard. The differences in measured isotope ratios $[n(^{34}S)/n(^{32}S)]$ for a given sample are reported as $\delta^{34}S$ values defined in Eqn. (1) below:

$$\delta^{34}S_{VCDT} = \frac{\left[\frac{n(^{34}S)}{n(^{32}S)}\right]_{SAMPLE} - \left[\frac{n(^{34}S)}{n(^{32}S)}\right]_{VCDT}}{\left[\frac{n(^{34}S)}{n(^{32}S)}\right]_{VCDT}} \quad (1)$$

where $[n(^{34}S)/n(^{32}S)]_{SAMPLE}$ is the measured $(^{34}S)/(^{32}S)$ ratio in the sample and $[n(^{34}S)/n(^{32}S)_{VCDT}]$ is the measured $(^{34}S)/(^{32}S)$ ratio in the standard IAEA-S-1. The primary isotope reference material for the $\delta^{34}S$ isotopic scale is the International Atomic Energy Agency's IAEA-S-1, which has a consensus value of −0.3‰ on the VCDT scale.[12,13]

Recently, a double spike (^{33}S-^{36}S) multi-collector thermal ionization mass spectrometry (MC-TIMS) technique has been used to measure $\delta^{34}S_{VCDT}$ in three international reference materials (IAEA-S-1, S-2, and S-3) and in low concentration snow pit samples from Greenland.[14–16] The MC-TIMS technique can be used as a relative-difference method (delta scale) providing an independent method from those typically used for stable isotope analysis of sulfur – gas source isotope ratio mass spectrometry (GIRMS) and multi-collector inductively coupled plasma mass spectrometry (MC-ICPMS). The double spike technique offers several advantages and benefits for sulfur isotope analysis beyond that of GIRMS and MC-ICPMS: (1) it employs an internal standard, which is intrinsically accurate because only isotopic ratios need to be measured, rather than an external standard; (2) it is free from spectral interferences (e.g. argides, nitrides, oxides, and hydrides); (3) because the mass spectrometric measurement is performed using MC-TIMS, the scale contraction problems common to GIRMS are

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RCM

eliminated; (4) sulfur concentration can be measured simultaneously; and (5) the blank can be evaluated.[14] Finally, the precisions obtained are typically equal to or better than those obtained using GIRMS and MC-ICPMS.[14,15] Methionine samples have been measured using this novel technique as a part of an inter-laboratory study (CCQM-P75) led by the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) in cooperation with the International Atomic Energy Agency (IAEA, Vienna, Austria) for the determination of δ^2H, $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, and $\delta^{34}S$ in methionine. The purpose of the inter-laboratory comparison was to assess measurement capabilities and the comparability of measurement results among recognized experts.

EXPERIMENTAL

Reagents

The preparation of the reducing solution used for the reduction of sulfate to hydrogen sulfide followed that described by Thode *et al.*[17] using HI (125 mL) (Sigma Aldrich, St. Louis, MO, USA), H_3PO_2 (61 mL) (Sigma Aldrich), and high-purity Optima HCl (205 mL) (Thermo Fisher Scientific, Pittsburgh, PA, USA). The mixed solution was refluxed under a nitrogen (N_2) stream (0.2 L/min) at 120°C for 3 h to remove any sulfur as H_2S. The As-NH_3 solutions were prepared from a saturated ammonia solution by bubbling high-purity NH_3 through a water scrubber and into chilled quartz-distilled water.[18] Both solutions were prepared by dissolving Standard Reference Material 83c (As_2O_3)[19] in saturated NH_3 solution. The first solution, which is used to trap the H_2S, contained an As concentration of 1000 μg/mL and the dilution solution was approximately 312 μg/mL. Silica gel was prepared by fusing high-purity quartz with sodium carbonate (Na_2CO_3) and washed with quartz-distilled water.[20] The silica gel was mixed with phosphoric acid (H_3PO_4) prepared from high-purity P_2O_5 and quartz-distilled water to yield a silica gel concentration of 20 μg/μL of 0.4 mol/L H_3PO_4. The silica gel and H_3PO_4 are premixed in a ratio designed to maximize signal level and stability.[21] High-purity nitric acid (HNO_3) (Seastar, Sidney, BC, Canada) and hydrochloric acid (HCl) (Thermo Fisher Scientific Optima) were used for all chemical processing steps.

Digestion method for quantitative analysis

The digestion method used for analysis is similar to that described in Paulsen and Kelly.[21] Three sample bottles of methionine (a commercially available L-methionine), containing approximately 200 mg each, were received from the IRMM and stored in a laboratory refrigerator at 4°C. Prior to analysis, the bottles were allowed to come to room temperature. Approximately 30 to 40 mg from each bottle was added by difference to a Carius tube along with 6 g of high-purity HNO_3. All sample weighings were carried out in a clean room on a calibrated five-place balance. The contents of the tube were then frozen in a solid CO_2/$CHCl_3$/CCL_4 mixture and the tubes sealed with an oxygen-natural gas torch. After warming to room temperature, the Carius tubes were placed inside steel shells along with 20 g of solid CO_2 for external pressurization, and then combusted at 240°C for 10 to 12 hours. The combustion step converts the sulfur in the methionine into sulfate. After cooling to room temperature, the tubes were vented and their contents transferred to new pre-cleaned 100 mL glass beakers. Sufficient high-purity NaCl (molar ratio Na/S ≈ 4) was added to prevent loss of sulfate during drying. The samples were heated to dryness and converted into the chloride form by the addition of 5 mL of high-purity HCl and heated to dryness again. This step was repeated two more times. The samples were then diluted in 5 mL high-purity HCl and 2 mL of 18MΩ MilliQ water (Modulab ModuPure Plus, Continental Water Systems Corporation, San Antonio, TX, USA). At this point the samples were transferred to 125 mL high-density polyethylene (HDPE) bottles where they were diluted to yield concentrations of approximately 100 μg S/g for two of the methionine bottles and 65 μg S/g for the remaining bottle.

Sample and standard preparation

The chemical preparation used for these measurements follows that described by Paulsen and Kelly[21] and Mann and Kelly.[14] Using a 5 mL plastic syringe, three samples each of approximately 1 g to 1.5 g, equaling approximately 100 μg S, were removed from each of the methionine solution bottles and were added by mass directly to 30 mL polycarbonate (Nalgene) bottles. Similarly, 0.3 to 0.4 g of sample, also equaling approximately 100 μg S, was taken from each of the standard solution bottles. Approximately 1.5 g of spike was added by weight to the polycarbonate bottles using a 3 mL plastic syringe followed by 2 mL of high-purity HCl and 2 mL of 18MΩ MilliQ water. Approximately 1 mole of the ^{33}S-^{36}S double spike was added for every 3 moles of sample/standard. Two additional samples of approximately 0.2 g, equaling approximately 10 μg S, were also taken for the assessment of the concentration for one of the methionine samples. Both the spike and the sample/standard are in solution and are of the same chemical form (SO_4^{2-}), thus eliminating potential heterogeneity associated with using the solid forms. It is possible that chemical processing of the samples could produce an isotopic fractionation artifact particularly if chemical yields were low and variable. To cover this possibility, samples were spiked before chemical processing; thus, any subsequent fractionation produced during processing would also fractionate the double spike in the same manner, preserving the true isotopic signature of the sample. The samples and standards were processed in groups of four: three samples/standards and one blank.

Reduction chemistry

All samples (standards, methionine samples, and blanks) were processed through the reduction procedure within 24 h of preparation again following Paulsen and Kelly[21] and Mann and Kelly.[14] A volume of 15 mL of the reducing solution was refluxed for 45 min at 120°C with nitrogen (N_2) gas injected through a sidearm (0.12 L/min) and cooled. The sample was then transferred to the reduction flask and refluxed for 45 min. The sulfate in the sample was reduced to H_2S and flushed out of the flask through a 10 mL distilled water trap and trapped in a 15 mL centrifuge tube containing 1 mL of the aqueous As-NH_3 trap solution. During the collection step the As-NH_3 solution was cooled in an ice bath

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to minimize loss of NH_3. The trapped sulfur was precipitated as arsenic sulfide (As_2S_3) with the addition of HCl. The precipitate was centrifuged for 10 min to remove the As_2S_3 particles from the solution. The supernatant was removed with a Pasteur pipette. The arsenic sulfide precipitate was then washed and centrifuged three times with 10 mL portions of 18 MΩ MilliQ water. The cleaned As_2S_3 was re-dissolved in the As-NH_3 dilution solution to yield a molar ratio of As/S = 2 and a [S] = 0.1 g/L. An important feature of this reduction chemistry is that it yields pure As_2S_3 for all samples, whether pure standards or samples of complex matrix.

Thermal ionization mass spectrometry

Mass spectrometric analyses were performed using a VG Sector-54 (IsotopX Ltd., Mansfield, MA, USA formerly VG Instruments) thermal ionization mass spectrometer (housed in the Isotope Geochemistry Laboratory in the Department of Geology at the University of Maryland, College Park, MD, USA) equipped with seven Faraday collectors and a 20-sample turret operated in the 'static' mode. The mass spectrometric procedure is based on the production of AsS^+ molecular ions from a single rhenium filament using silica gel to enhance ionization.[14] The volatility of As_2S_3 (As_2S_3 vaporizes at 700°C at 1 atm.) is reduced by mixing silica gel with the sample on the filament. Rhenium filaments were fabricated from zone-refined Re ribbon (H. Cross Co., Moonachie, NJ, USA – thickness: 0.003048 cm (0.0012″), width: 0.0762 cm (0.030″), length: 2.54 cm (1″)) and outgassed initially for 5 min at 2 A and 1.33×10^{-3} Pa (10^{-6} Torr) followed by two 2 min cycles of 5 s flashings at 3.5 A at the same pressure. The filaments were stored for approximately 1 week before use. The samples were loaded on the Re filaments in a laminar flow hood. Details of the loading procedure are described in Paulsen and Kelly.[5] In brief, the silica gel/H_3PO_4 solution is placed in an ultrasonic bath for 10 min to facilitate the suspension of the silica gel particles. The solution is then shaken vigorously prior to each loading and 5 μL are placed in the center of the filament using 5 cm length, 0.0762 cm (0.030″) i.d. intramedic polyethylene tubing attached to a 21-gauge hypodermic needle affixed to a Hamilton microliter syringe. A 0.9 A current was applied until the solution was dry. Then 15 μL (≈1.5 μg S) of the As_2S_3 sample solution was added as droplets to the dried silica gel and evaporated to dryness at 1.6 A. The current was then increased and the sample dried at a red glow for about 1 to 2 s. The sample solutions were aged for a minimum of 12 h before loading for optimum signal intensity and stability. It is important to note that the same amount of As_2S_3 sample solution is loaded each time; therefore, the signal intensities should be the same thus yielding similar precisions for the $\delta^{34}S$ measurements regardless of the sample amount processed.

All samples were placed into the mass spectrometer turret immediately upon completion of loading to minimize exposure to room atmosphere. After pump down, the pressure in the source was further reduced to below 2×10^{-4} Pa (2×10^{-7} mbar) with the addition of liquid N_2 to the source cold finger. The filament was initially heated at a current of approximately 1.8 A (≈700°C) for 5 min and then incrementally heated to a final current of approximately 2.1 A (≈1000°C). The AsS^+ signal intensity was typically 1 to 3.5 V at mass 107 ($^{75}As^{32}S$) and 60 to 125 mV at 109 ($^{75}As^{34}S$) (10^{11} Ω feedback resistor) after focusing for maximum intensity. Data collection began approximately 20 to 30 min after commencing filament heating. Measurements of the $^{75}As^{32}S/^{75}As^{33}S$, $^{75}As^{34}S/^{75}As^{33}S$, and $^{75}As^{36}S/^{75}As^{33}S$ ratios were made using the axial and three off-axial collectors Ax, H1, H2, and H4 for ion currents $^{75}As^{32}S$, $^{75}As^{33}S$, $^{75}As^{34}S$, and $^{75}As^{36}S$, respectively. Five data blocks were collected, each block consisting of 10 integrations of all masses using an integration time of 10 s on peak. Background signals were read before each set using an integration time of 10 s. Typical run time was about 25 min. An integration time of 10 s has proven to be more than adequate for signal intensities above 15 mV on mass 109 ($^{75}As^{34}S$).

Data reduction

The double spike method has been used for the rigorous correction of instrumental mass fractionation associated with TIMS measurements and permits natural fractionation to be separated from the instrumental fractionation.[22–35] Application of the technique for other elements has repeatedly demonstrated that marked improvements in measurement precision are achievable, which have permitted fractionation effects as small as 0.1‰ to be observed. In this work the $^{32}S/^{34}S$ natural isotope ratios are calculated from the mass spectrometric measurements using an iterative procedure.[14] In this calculation the instrumental fractionation factor (α) and the natural sample isotope ratios are successively refined until convergence. Sulfur isotope fractionation during thermal ionization follows Rayleigh fractionation[14] similar to what is observed for Ca and Se.[22,23] Thus the exponential fractionation law was employed for the correction of the instrumentally fractionated measured ratios as this law most closely models Rayleigh fractionation.

The $\delta^{34}S$ values obtained from the iterative calculation scheme above are not the true $\delta^{34}S$ values but may be biased due to contribution from the blank. The method used for blank correction and determination of the blank-corrected or true $\delta^{34}S$ value was similar to that described in Hayes.[36] Full details of the data reduction procedure are discussed in Mann and Kelly.[14]

Spike calibration

The isotopic composition of the ^{33}S-^{36}S double spike (DS) was determined using sample sizes of approximately 290 μg S. The sample size was chosen to minimize the effect of the blank, which is typically less then 0.1 μg based on our accumulated blank record covering 3 years ($n \approx 60$), and to minimize the amount of spike used. The DS composition was calibrated against the absolute value for IAEA-S-1 ($^{75}As^{32}S/^{75}As^{34}S = 22.6504$[37]). The absolute value was determined by IRMM using synthetic isotope mixtures for calibration prepared gravimetrically from high-purity Ag_2S enriched in ^{32}S, ^{33}S, and ^{34}S. All materials were converted into SF_6 gas and the isotopic ratios were measured as the SF_5^+ species using a special gas source mass spectrometer (IRMM's Avogadro II amount comparator) equipped with a molecular flow inlet system.[37] If the DS

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isotope composition is calibrated correctly, repeated measurements of the spiked standard should yield an average $\delta^{34}S$ within the absolute reference value provided by IRMM of $-0.30‰ \pm 0.12‰$ relative to Vienna Canyon Diablo Troilite (VCDT).[14] Calibration of the DS composition based on the absolute value for IAEA-S-1 yields a double spike composition that is absolute and traceable to the ratio of two SI quantities.

The concentration of the double spike was determined by calibrating it against a dilute solution prepared gravimetrically from Standard Reference Material (SRM) 3154 (Sulfur Spectrometric Solution). This SRM was certified coulometrically as well as gravimetrically and the combined uncertainty on the certificate is 0.3%, relative.[38] The assay standard was serially diluted to a final concentration of approximately 100 μg/g. This was done in quadrature, four separate preparations, so that any subsequent aliquoting or dilution errors could be readily identified. Approximately 0.6 to 0.75 g of each final solution was weighed into 30 mL polycarbonate bottles and mixed with 0.5 to 0.8 g of the ^{33}S-^{36}S spike solution together with ≈ 2 mL of high-purity HCl and about 2 mL of 18 MΩ MilliQ water. Samples were taken from each of the four bottles to produce six calibrant solutions. The solutions were shaken vigorously and stored overnight to assure sample-spike equilibration.

Blanks

A total of six blanks were prepared by adding 2 mL of 18 MΩ MilliQ water and 2 mL of high-purity HCl to 30 mL polycarbonate bottles followed by the addition of 0.5 g of spike. The blanks were processed in a manner similar to the samples (i.e. sample preparation and reduction chemistry). Two blanks were also measured for blank correction of the spike calibration samples.

RESULTS AND DISCUSSION

$\delta^{34}S$ and concentration measurements

A summary of the $\delta^{34}S$ results for the methionine samples is given in Table 1a. The results reported include the expanded uncertainty (U) determined using k = 2.3. Table 1b and Fig. 1 provide a summary of the data for the three individual bottles of methionine together with the expanded uncertainties for each. The uncertainties were determined in accordance with ISO guidelines.[39] A description of the specific components of uncertainty for these materials is given in Table 2.

Table 1a. Summary results for the sulfur isotope composition ($\delta^{34}S$) for methionine

Methionine	Sulfur isotope composition $\delta^{34}S$ (‰) relative to VCDT
Value	10.34
Combined standard uncertainty	0.048
Effective degrees of freedom	9
Coverage factor (k)	2.31
Expanded uncertainty (U)	0.11

Table 1b. Sulfur isotope composition ($\delta^{34}S$) results for each bottle of methionine

Sample ID	$\delta^{34}S$ (‰)	1σ	$2\sigma_m$[a]	U[b]
Bottle #1	10.37	0.25	0.071	
Bottle #1	10.19	0.40	0.11	
Bottle #1	10.13	0.30	0.086	
Mean $\delta^{34}S$ value (n = 3)	**10.23**	**0.13**	**0.14**[b]	**0.25**
Bottle #2	10.25	0.31	0.088	
Bottle #2	10.47	0.23	0.064	
Bottle #2	10.40	0.40	0.11	
Mean $\delta^{34}S$ value (n = 3)	**10.37**	**0.11**	**0.13**[b]	**0.23**
Bottle #3	10.38	0.40	0.11	
Bottle #3	10.45	0.20	0.056	
Bottle #3	10.32	0.19	0.054	
Mean $\delta^{34}S$ value (n = 3)	**10.38**	**0.07**	**0.077**[b]	**0.16**

[a] k = 2 (n = 49) for the nine individual runs.
[b] k = 3.2 (n = 3)

The value determined for this material was $10.34‰ \pm 0.11‰$ (n = 9). The quantitative analyses of the three bottles of methionine resulted in highly precise data with an expanded uncertainty of 0.11‰ for k = 2.3 (n = 9) (Table 1a). The standard errors for the three measurements for bottles 1, 2, and 3 were 0.14‰, 0.13‰, and 0.08%, respectively (Table 1b). The error associated with the sample measurement was the largest of all the elements in the uncertainty budget. The next largest factor was the measurement uncertainty associated with the calibration of the double spike. The spread in the data shown in Fig. 1 is greater than expected from sampling a single parent population, where 95% of the data, if compared as 95% confidence interval (CI) as we did, are expected to cross the mean. This is probably the result of small changes in and among the detectors between measurements. Two additional measurements on bottle 3 were made using a factor of 10 less sample (10 μg) with the results being $10.62‰ \pm 0.24‰$ (1σ) and $10.63‰ \pm 0.28‰$ (1σ). Both results fall within in our uncertainty reported, showing the capability of the technique for measuring small sample sizes.

One of the major advantages of the double spike technique, in addition to measuring the natural isotopic composition, is that the sulfur concentration can be determined simultaneously by isotope dilution (ID).[14,16] In this case the spike isotope used is ^{33}S. The results are listed in Table 3. Although this material is not certified for concentration, the uncertainty in the concentration determinations for each of the three

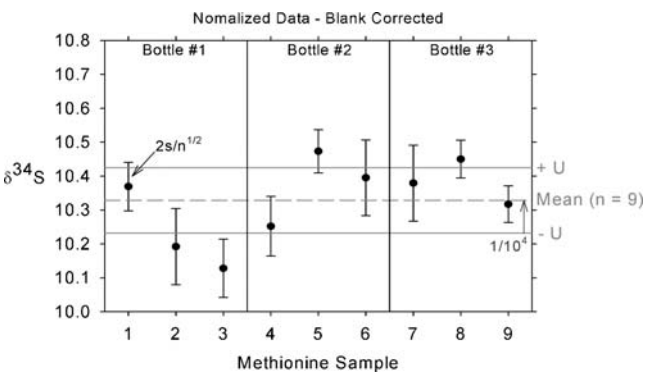

Figure 1. Graph of methionine data for three mass spectrometric determinations from each bottle.

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Table 2. Components of uncertainty

Uncertainty	Basis	Type	DF
MC-TIMS measurements	This is derived from the RSD of the individual sample measurements after corrections for blank have been applied. This number is divided by the square root of N, the number of independent samples measured. N = 9.	A	8
DS spike calibration	This is derived from the RSD of the individual calibration mix measurements after all corrections for blank have been applied. This number is divided by the square root of N, the number of independent samples measured. N = 4.	A	3
Calibrant for DS	The number for IAEA-S-1.[37]	B	∞
Sulfur isotope composition	The combined uncertainty on the nine pooled mixes from the three different methionine bottles is square root of the sum of the uncertainty (1σ) from the IAEA-S-1 calibrant runs (the square of 0.056 divided by 4) added to the square of the uncertainty (1σ) of the mean of the nine mixes (the square of 0.117 divided by 9) and yields u_c.	Combined	9
	The expanded uncertainty (U), using a 95% confidence interval, was calculated by multiplying the combined standard uncertainty by k = 2.3 (NIST) or 2.0, as appropriate (IRMM).	Expanded ($p = 0.05$)	9

bottles was low, with relative standard deviations (RSDs) of 0.15% (n = 3), 0.26% (n = 3), and 0.32% (n = 5), for bottles 1, 2, and 3, respectively. These data highlight the ability of the technique to measure both the natural composition and the concentration simultaneously with high accuracy and precision on sample sizes varying by a factor of 10.

Spike calibration

The atom abundances, in percent, and concentration for the DS are given in Table 4a. The results for the four different mixes of the DS with IAEA-S-1 for the calibration of the DS for isotope composition are shown in Table 4b and Fig. 2. The mean of the measurements for IAEA-S-1 (−0.29‰ ± 0.056‰ (1σ), n = 4) is consistent with the reference values reported by NIST, the 8th Working Group (consensus), and IRMM (−0.30‰ ± 0.12‰ (1σ)) as well as with the value previously measured by us (−0.31‰ ± 0.13‰ (1σ)).[13,14,37,40] The results of the calibration for concentration are listed in Table 4c. Figure 3 is a plot of the calibration results listed in Table 4c. The S concentration for the DS was 0.656 ± 0.00109 μmol/g (1σ) based on six mixes. The RSD for these six determinations was 0.068%.

Table 3. Concentration results for methionine samples

Sample identification	[S] μg/g
Bottle #1	77.28
	77.51
	77.34
Blank	0.017 μg S
Average	77.38
1σ	0.12
N	3
RSD	0.15%
Sample identification	**[S] μg/g**
Bottle #2	88.46
	88.04
	88.08
Blank	0.023 μg S
Average	88.19
1σ	0.23
N	3
RSD	0.26%
Sample identification	**[S] μg/g**
Bottle #3	56.78
	56.41
	56.44
	56.49
	56.30
Blank	0.029 μg S
Average	56.48
1σ	0.18
N	5
RSD	0.32%

Table 4a. S Concentration and atom abundances in % of the double spike as used in the data reduction

Spike	[S] (μmol/g)	^{32}S	^{33}S	^{34}S	^{36}S
^{33}S-^{36}S	0.656 ± 0.0011	0.38	48.96	0.031	50.63

Table 4b. Double spike composition calibration and control results for IAEA-S-1

IAEA-S-1 (RM 8554)	$\delta^{34}S$ (‰)
Reference value from NIST[a]	*−0.30*
Consensus value[b]	*−0.30*
Reference value from IRMM[c]	*−0.30 ± 0.12 (1σ)*
Previously measured value[d]	*−0.31 ± 0.13 (1σ)*
	−0.22
	−0.31
	−0.36
	−0.28
Average	−0.29
1σ	0.056
$2\sigma_m$ (k = 2)	0.056

[a] Value from the Report of Investigation for Reference Materials 8553-8557.[40]
[b] Value from the Report of the Sulfur Isotope Working Group.[13]
[c] Ref. 37.
[d] Value previously measured using the double spike technique.[14] Based on n = 4.

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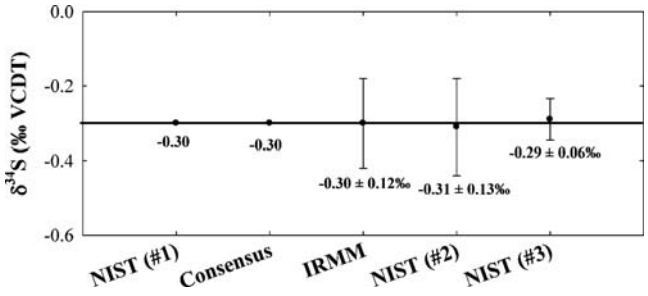

Figure 2. Graph of spike calibration data listed in Table 4b. NIST (#1) is the reference value (defined) taken from the Report of Investigation, NIST (#2) is the previously measured (June 2004) value using the double spike, and NIST (#3) is the value determined in this study (June 2006). Error bars are 1σ. The solid line represents the consensus value which is the current accepted value for the sulfur standards.

Table 4c. Double spike concentration calibration results. Four different stock solutions were prepared and used in the calibration. All spike concentration numbers are blank corrected

Sample identification	[S] μmol/g
Mix 1a	0.654
Mix 1b	0.656
	0.656
Blank	0.0945 μg S
Mix 2a	0.656
	0.657
Mix 2b	0.657
Blank	0.0354 μg S
Spike concentration average	**0.656**
1σ	**0.0011**
N	**6**
RSE	**0.068%**

Blank measurements

The absolute sulfur blanks measured during the course of this study ranged from 0.0023 to 0.011 μg S. Three blanks each were processed with the three samples from each bottle as well as the control standards. As each group of samples was handled as a unit, the data from each unit were corrected with the corresponding blank associated with that unit. The blank correction was 0.0030%, 0.0026% and 0.0042% for bottles 1, 2 and 3, respectively.

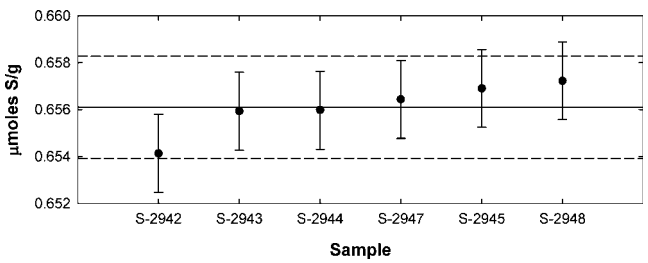

Figure 3. Graph of spike calibration data listed in Table 4c. The solid line represents the mean of the data while the dashed lines represent $\pm 2\sigma_m$ of the mean. Uncertainties on the individual measurements are ± 0.17% rel.

All reported $\delta^{34}S$ values for the methionine samples have been corrected for blank. Previous experiments indicate that the blank is derived from the silica gel and the As-NH_3 dilution solution; thus the variability observed in the blanks is coming from the loading portion of the procedure. Hence, the blank composition of +36‰ (n = 2) used in the Hayes[36] correction scheme was directly measured, by adding only silica gel and As-NH_3 solution to the filament.[14] The same silica gel and As-NH_3 solution were used for all the standards measured; thus, the blank composition should be constant. The uncertainty in the composition may be quite large due to the limited ability to directly measure the composition of the blank on such small samples. Thus, a sensitivity analysis was performed by changing the composition of the blank (by ± 50‰). However, because the natural sample sizes used in this study were relatively large (sample-to-blank ratios were greater than 9000) the effect of blank composition uncertainty is quite small (<0.01‰).

Controls

Two different control standards (IAEA-S-1 and S-2) were run concurrently with the methionine samples and the results are shown in Tables 4b and 5a and Figs. 2 and 4. The IAEA-S-1 samples used for calibration of the spike can be viewed as control samples. The mean of the four determinations of IAEA-S-1 (−0.29‰ ± 0.056‰ (1σ)) shows no discernible

Table 5. Control standards run with the methionine samples

IAEA-S-2 (RM 8555)	$\delta^{34}S$ (‰)
Reference value[a]	*22.7 ± 0.2*
Consensus value[b]	*+22.66 ± 0.13 (1σ)*
Previously measured value[c]	*+22.60 ± 0.06 (1σ)*
	22.55
	22.63
	22.65
AVG	22.61
1σ	0.052
$2\sigma_m$ (k = 2)	0.060

[a] Value reported by IAEA.[41]
[b] Refs. 13 and 41.
[c] Value previously measured using the double spike technique.[14] Based on n = 6.

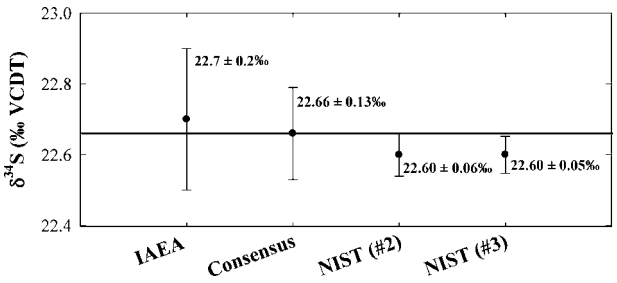

Figure 4. Graph of control data for IAEA-S-2 listed in Table 5. NIST (#2) is the previously measured (June 2004) value using the double spike and NIST (#3) is the value determined in this study (June 2006). Error bars are 1σ. The solid line represents the consensus value which is the current accepted value for the sulfur standards.

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difference from the reference values reported.[13,37,40] In addition, the mean for IAEA-S-2 (+22.61‰ ± 0.052‰ (1σ)) is also consistent with the consensus value reported by IAEA (+22.66‰ ± 0.13‰ (1σ))[14,41] and with our previously determined value (+22.60‰ ± 0.06‰ (1σ)) using the double spike technique.[13] The reference value listed for IAEA-S-2 is taken from the working group consensus value.

CONCLUSIONS

The isotope composition and concentration results demonstrate the high accuracy and precision of the DS-MC-TIMS technique. The DS-MC-TIMS technique is an internal correction method where the only known potential bias is the fractionation law (linear, power, or exponential) used to model and correct for instrumental fractionation. It follows that many of the biases associated with the GIRMS and ICP-MS techniques are thus eliminated. It is also important to note that these $\delta^{34}S$ measurements include a correction for blank, which must be measured to assess the accuracy of the $\delta^{34}S$ data. In previous studies there is limited to no mention of blanks and although the blanks were negligible in this study this potentially significant bias and uncertainty typically has been ignored. Therefore, mass spectrometric measurement by TIMS is likely to be a better representation of the true value.

DISCLAIMER

Certain commercial equipment, instruments, or materials are identified in this paper in order to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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