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RATIONALE: Precise analysis of four sulfur isotopes of sulfate in geological and environmental samples provides the means to extract unique information in wide geological contexts. Reduction of sulfate to sulfide is the first step to access such information. The conventional reduction method suffers from a cumbersome distillation apparatus system, long reaction time and large volume of the reducing solution. We present a new and simple method enabling the process of multiple samples at a time with a much reduced volume of reducing solution.

METHODS: 1 mL reducing solution made of HI and NaH_2PO_2 was added to a septum glass tube with dry sulfate. The tube was heated at 124 °C and the produced H_2S was purged with inert gas (He or N_2) to go through gas-washing tubes and then collected by NaOH solution. The collected H_2S was converted to Ag_2S by adding $AgNO_3$ solution and the co-precipitated Ag_2O was removed by adding a few drops of concentrated HNO_3.

RESULTS: Within 2-3 hours, a 100 % yield was observed for samples with 0.2 to 2.5 µmol Na_2SO_4. The reduction rate was much slower for $BaSO_4$ and a complete reduction was not observed. International sulfur reference materials, NBS-127, SO-5 and SO-6, were processed with this method, and that the measured against accepted $\delta^{34}S$ values yielded a linear regression line which had a slope of (0.99 ± 0.01) and a R^2 value of 0.998.

CONCLUSIONS: The new methodology is easy to handle and allows to process multiple samples at a time, and was demonstrated with good reproducibility in terms of H_2S yield and for further isotope analysis. It is thus a good alternative to the manual

conventional method, and especially when processing samples with limited amount of sulfate available.

Stable sulfur isotopes have been widely used to trace a range of biogeochemical processes [1]. Especially, in 2000, the discovery of the mass-independent isotopic fractionations of sulfur isotopes (S-MIF) in sulfate and sulfide in Archean rocks [2] have shown the potential of the S-MIF signals on tracking the oxygenation of the atmosphere 2.4 Gy ago [3], and the geochemical evolution of Mars [4]. The S-MIF signals in ice-core sulfate have also been observed and demonstrated to be useful to track sulfur cycle in today's stratosphere and serve as a unique proxy of large volcanic eruptions that inject sulfur into the stratosphere and thus have global climate impacts [5-8]. Multiple sulfur isotope compositions can also help to constrain the oceanic sulfur cycle (e.g., [9, 10]).

To access the S-MIF signals, precise analysis of the four sulfur isotopes (^{32}S, ^{33}S, ^{34}S and ^{36}S) is necessary. The isotopic results are expressed as $\delta^{3x}S = {}^{3x}R_{sample}/{}^{3x}R_{CDT}-1$, where x = 3, 4, and 6, and the δ values are calculated using the CDT standard. The S-MIF values are then defined by:

$$\Delta^{33}S = \delta^{33}S - [(\delta^{34}S + 1)^{0.515} - 1]$$
$$\Delta^{36}S = \delta^{33}S - [(\delta^{34}S + 1)^{1.90} - 1]$$

The isotopic analysis is conventionally done by reducing sulfate (SO_4^{2-}) to sulfide (H_2S), converting H_2S to silver sulfide (Ag_2S), and fluorinating Ag_2S to sulfur hexafluoride (SF_6) for isotopic composition analysis by isotope ratio mass spectrometry [2, 6, 11, 12]. In the literature, the reduction from SO_4^{2-} to H_2S is mainly achieved by two different reducing agents, Tin(II) (Sn^{2+}) solutions and hydroiodic acid (HI) hypophosphorous acid (H_3PO_2) mixtures [13-15]. The Sn^{2+} solution is mainly applied for solid samples (e.g.,

minerals) with an optimum reaction temperature between 280-300 °C, and the HI acid reducing solution can be applied to aqueous samples at 100-125 °C [14]. Currently, the most applied reducing method used in sulfur isotope geochemistry follows the reducing agent recipe (500 mL concentrated HI, 816 mL concentrated HCl, and 245 mL 50 % H_3PO_2) of Thode et al. [16] and a distillation apparatus similar to that described in Forrest and Newman [17].

In the Thode et al. [16] reducing solution, high concentrations of HI seem to be the most important component of the reducing agent for complete sulfate reduction, and the presence of H_3PO_2 or NaH_2PO_2 fastens the reduction speed by maintaining a high hydroiodic acid to iodine ratio which is one of the factors favoring the reduction [14, 18]. HCl acid is only of secondary importance and its presence is suggested to be used to increase the acidity and volume, and reduce the use of relatively expensive HI [13, 19]. However, Gustafsson [20] found the presence of water is detrimental for the reduction because water tends to dilute and thus lower the concentration of HI, and at lower HI concentration, side product (i.e., SO_2 and elemental S) will be formed [18]. In this regard, mixing 50 % H_3PO_2 and concentrated HCl to concentrated HI may have draw backs for the reduction efficiency, because both H_3PO_2 (50 %) and concentrated HCl (37 %) acids comprise more than half of water by weight. To avoid additional water in the reducing solution, the H_3PO_2 acid can be replaced with dry NaH_2PO_2 salt, and HCl acid can be omitted. Gustafsson [20] and Davis and Lindstrom [18] have used a reducing solution containing only HI acid (57 %) and NaH_2PO_2 salt, and found good reduction yield. In particular, Davis and Lindstrom [18] found an optimum composition of reducing solution for complete and fast sulfate reduction is 0.13 g NaH_2PO_2 in 1 mL HI (57 %). In these

studies, aqueous sulfate samples were processed and a cumbersome distillation apparatus was used.

Summarized from the literature, it seems that the best composition of the reducing solution would be a mixture of 0.13 g NaH_2PO_2 in 1 mL HI (57 %), and water should be limited in the reduction experiment. The latter suggests dry sulfate samples are a better choice as the starting material. Typically, barium sulfate ($BaSO_4$) is the preferred sulfate form used for the four sulfur isotope analysis because it is the natural form found in major geological samples or can be readily prepared from natural samples containing soluble sulfate (e.g., sea water) by precipitation with excess $BaCl_2$ solution. $BaSO_4$ has very low solubility (≈ 0.02 mg/L at 20° C) and this may inhibit the reaction efficiency and speed, especially when the volume of the reducing solution is small. Alternatively, soluble sulfate in natural samples can also be extracted and purified by other methods such as using an ion-exchange resin [21] and which can lead to dry Na_2SO_4 by evaporating the eluent. We thus conducted tests with both $BaSO_4$ and Na_2SO_4 to explore the reaction efficiency of the reduction process with respect to different sulfate forms. In this study, we present a series of experiment using a reducing solution comprising NaH_2PO_2 and HI (57% by weight) to process dry sulfate samples (both Na_2SO_4 and $BaSO_4$). To avoid the cumbersome distillation apparatus, we tested a simple flow system with only sealed glass tubes connected by PEEK tubes and explored the possibility to process multiple samples at a time. The reproducibility for H_2S yield and for further sulfur isotope analysis is reported.

EXPERIMENTAL

Reagents

The new reducing solution was made of 100 mL concentrated hydriodic acid (HI, 57 % by weight,) and 13 g sodium hypophosphite (NaH_2PO_2). The reducing solution should be made in a fume hood, where 100 mL HI and 13 g NaH_2PO_2 were added to a flask. The flask was placed on a hot plate magnetic stirrer and a magnetic stir bar was used to mix the liquid and the salt. Because HI is easily oxidized by O_2, helium (He) or another inert gas stream (e.g., N_2) was introduced by a PEEK tube to the flask to purge the mixture. While purging with He, the hot plate was turned on and the temperature was set at 130 °C. The flask was heated at 130 °C for at least one hour to reduce any sulfur compounds into H_2S (that was flushed away from the reagents) and to reduce traces of I_2 (in the form of I_3^-) into I^- by NaH_2PO_2. The solution started with deep color (I_3^-) and became colorless with time. After heating for 1 hour, the solution was allowed to cool down under the He stream and then stored in a sealed brown bottle. The reducing solution may be oxidized overtime, which is indicated by light yellow color and the color may get deeper depending on the degree of oxidation.

Different from what can be found in the literature, in this study, we used sodium hydroxide (NaOH, 0.1 M) as the trapping solution to collect the reducing product H_2S. Conventional trapping solution, cadmium (or zinc) acetate ($Cd(CH_3CO_2)_2$, 0.1 M) and/or silver nitrate ($AgNO_3$, 0.01 M) were also made and used, and the results were compared with that from the NaOH trapping solution. As detailed below, using NaOH as the trapping solution allows direct quantification of sulfur concentration by UV absorption spectroscopy, which, in terms of the yield quantification, is faster and more reliable than gravimetric techniques.

Apparatus

The reduction train is sketched in Figure 1. The main parts of the apparatus are four 15 mL glass tubes each with nitrile/PTFE septum and a block heater. The reaction tube 'a', two gas washing tube 'b1' and 'b2' and the collection tube 'c' were connected with PEEK tubes (1/16" ID) directly through the septum. Alternatively, a drying cartridge filled with potassium perchlorate ($KClO_4$) and a cryogenic trap (which its internal temperature can be controlled between -200 °C and -80 °C) could be placed between the b2 trap and the collection tube to test the possibility of using pure H_2S as the working gas for isotope analysis. The aim of the drying cartridge and the cryogenic trap is to purify H_2S without any loss. Dry sulfate sample (i.e. Na_2SO_4) and 1 mL reducing solution were introduced in glass tube 'a', which was placed on a block heater and purged with He flow for 20 minutes before turning on the heater to a temperature set at 124 °C. The purge before the heating is necessary to remove trace I_2, especially when the reducing solution has a light yellow appearance overtime due to slight oxidation.

A He tank supplied the He gas. In practice, we had a home-made flow distributor to distribute the He flow to eight flows, as shown in Figure 2. Each flow was then guided to an individual reduction train, and the flow rate (*~ 2 mL/min*) of each reduction train was controlled by a micro-flow meter (ref: P-446, IDEX Health & Science, Sainte-Foy-La-Grande, France). In this way, multiple samples can be processed simultaneously.

Testing samples

We used the above-mentioned reducing solution and apparatus to process dry sulfate samples in the forms of barium sulfate ($BaSO_4$) and sodium sulfate (Na_2SO_4). Na_2SO_4 samples were made from 1 mM Na_2SO_4 solution (0.142 g Na_2SO_4 in 1 L Milli-Q water, Millipore SAS, Molsheim 67120, France), and then a desired volume of the

Na_2SO_4 solution (e.g., 0.2 or 0.5 mL, equivalent to 0.2 or 0.5 μmol SO_4^{2-}) was added to a pre-cleaned reaction tube. The reaction tube was allowed to completely dry in a 100 °C oven, and the sample was then stored for later use.

In order to make $BaSO_4$ samples, a desired volume (e.g., 0.2 or 0.5 mL) of the 1 mM Na_2SO_4 solution was added to the reaction tube, and then a drop of 1 M HCl solution was added to the same tube to remove any carbonate in the solution. After overnight, a drop of 0.1 M $BaCl_2$ solution was added to the reaction tube to precipitate $BaSO_4$. After $BaSO_4$ precipitates, the sample was processed differently. For one set of $BaSO_4$ samples, they were just dried completely in an over at 100 °C, so the dry samples contain $BaSO_4$, $BaCl_2$ and NaCl. We termed this set of samples $BaSO_4$-EB ($BaSO_4$ with excess $BaCl_2$). For the other set of $BaSO_4$ samples, they were centrifuged and the supernatant was removed. The remained solids were rinsed with Milli-Q water and then separated from the rinsing water by centrifuge. This step was repeated three times before the sample was placed in the oven to dry. This set of samples was termed P-$BaSO_4$ (pure $BaSO_4$).

In addition, international reference materials (in the form of $BaSO_4$), IAEA-SO-5 ($\delta^{34}S$ = (0.5 ± 0.2) ‰), IAEA-SO-6 ($\delta^{34}S$ = (-34.1 ± 0.2) ‰) and NBS-127 ($\delta^{34}S$ = (20.3 ± 0.4) ‰) were prepared by weighing ~ 0.5 mg of the $BaSO_4$ standards to reaction tubes. After reduction, these samples were further converted to SF_6 for isotope analysis. We note the reference materials were not weighed precisely because of the capability of our balance (0.1 mg precision). However, the purpose of processing these samples is to test potential sulfur isotope fractionation during the reduction, rather than assessing the reduction yield (which can be assessed from the samples made from drying Na_2SO_4

187 solution with accurate measurement of sulfur content or precipitating $BaSO_4$ from the
188 same Na_2SO_4 solution).

189 **Quantification**

190 The yield of the reduction from sulfate (SO_4^{2-}) to sulfide (S^{2-}) can be directly
191 assessed by determining the quantity of H_2S collected in the NaOH trapping solution.
192 Hydrogen sulfide (H_2S) solution is known to absorb ultraviolet light with a peak
193 absorbance at 230 nm [22, 23]. Guenther et al. [22] have shown that in alkaline solutions with
194 pH > 8, H_2S is present nearly 100 % in the form of bisulfide ion (HS^-), and found that at
195 pH ~ 8, ultraviolet determination of HS^- yields are accurate because precise estimates of
196 total sulfide concentration in the solution can be achieved. Thus, with NaOH as the
197 trapping solution, the yield of the reduction can be directly assessed by measuring HS^- in
198 the solution with optical methods. In comparison, the conventional trapping solution
199 (cadmium acetate or silver nitrate) collects H_2S as a precipitate, which makes it difficult
200 to directly quantify the reduction yield.

201 In this study, we used an ultraviolet spectrophotometer (model 6850, Jenway,
202 Staffordshire, UK*)* to determine the concentration of H_2S in the NaOH trapping solution.
203 The calibration standards were made from mixing sodium sulfide nonahydrate
204 ($Na_2S{\cdot}9H_2O$, > 99.99% purity, Sigma-Aldrich, St. Louis, MO, U.S.A.) with 0.1 M NaOH
205 solution. Few $Na_2S{\cdot}9H_2O$ crystals were quickly rinsed on kimwipes® disposable wipers
206 to remove surface oxidation products, dried and weighed directly. A stock solution of
207 0.01 M HS^- was made by mixing 0.0125 g of pre-cleaned $Na_2S{\cdot}9H_2O$ in 5 mL 0.1 M
208 NaOH solution. A set of working standards, 0.0 μM, 20 μM, 50 μM and 100 μM were
209 then made by diluting 0, 0.02, 0.05 and 0.1 mL of the stock solution into the adequate

volume of 0.1 M NaOH to reach 10 mL standard solution. The stock solution should be stored in a sealed brown bottle and flushed with He before storage, since sulfide is easily oxidized by O_2 once in contact with air. Even flushed before storage, we noticed significant loss of sulfide from the stock solution after two to three days. Guenther et al.[22] made the stock solution in a glass aspirator bottle purged with N_2, and stated the solution should be stable for about 1-2 weeks. In practice, we made fresh stock solution once every two days, and working standards every day.

Procedure

Prior to the reduction, all glassware, caps, septum and PEEK tubes were cleaned with Milli-Q water. The PEEK tubes have to be flushed to make sure there is no water left inside of them, otherwise the water will block the flow of the carrier gas in the reduction line.

In a fume hood, 1 mL reducing solution was added to a pre-prepared reaction tube with known amount of dry sulfate. In the reaction tube, the reducing solution was purged with He for 20 minutes at room temperature to remove any I_2 and O_2. The gas washing tubes (b1 and b2 in Figure 1), and the collection tube (c in Figure 1) were prepared by adding 12 mL Milli-Q water and 12 mL 0.1 M NaOH, respectively. After the reducing solution was purged for 20 minutes, the reduction train was assembled (Figure 1) and the reaction tube was placed in the block heater and heated at 124 °C. At lower temperatures the reduction speed will be slow, while if the temperature is too high, excessive amount of phosphine (PH_3) will be produced from the decomposition of NaH_2PO_2 [14]. For the alternate set up, the drying agent was in-line with the cryogenic system, the latter was set at -200° C to trap the products of the reaction. When the reaction was over, the

temperature of the cryogenic trap was raised to -120° C from which the produced H_2S was released and trapped in the collection tube.

The collection tube was removed from the reduction train after the reaction was done. The concentration of H_2S in the trapping solution was firstly measured by the ultraviolet spectrophotometer as described in section 2.4, in order to assess the yield. After that, 1 mL 0.01 M $AgNO_3$ was added to the collection solution to precipitate Ag_2S and Ag_2O. After gently shaking, a few drops of concentrated HNO_3 (68 %) were added to the suspension. With thoroughly shaking, Ag_2O was dissolved and only Ag_2S remains in the solid phase. The tube was allowed to settle down, and a plastic laboratory dropper was used to remove the supernatant. The solid was then rinsed with Milli-Q water for three times, transferred to an aluminum boat and dried for fluorination.

Isotope analysis

To explore potential sulfur isotope fractionation during the reduction, we processed international sulfate reference materials, IAEA-SO-5, IAEA-SO-6 and NBS-127, following the above-mentioned procedure in section 2.5. The reference materials were weighed approximately 0.5 mg and added to the reaction tube. The reaction was stopped after ~ 5 hours.

After converting to Ag_2S as described in section 2.5, the reference materials were shipped to the Stable Isotope Geochemistry Laboratory at IPG-Paris (Institut de Physique du Globe) for sulfur isotope analysis. At IPG, the samples were dried and then transferred to aluminum boat and weighed. Due to the small quantity (~ 0.3 mg Ag_2S or less) of the sample, we found it is very difficult to transfer the dry Ag_2S from the collection tube to the Al boat. Alternatively, we transferred the solid with a small amount of water together

from the tube to the Al boat, and then dried the samples. Under this circumstance, we found that after drying, the inside wall of the Al boat became light-brown in color, and the mass of the dried Al boat with sample exceeded the sum of the sample and the Al boat, indicating the gain of extra mass during the drying process. This is likely due to the development of a thin layer of Al_2O_3 on Al metal surface when Al contacts with water at the drying temperature (70 °C). This is consistent with the observation that, after drying an Al boat with Milli-Q water at 70 °C, a brown layer on the inner surface of the Al boat was formed and the mass of the Al boat was increased . Nevertheless, the results of fluorination yields and sulfur isotopic analysis suggested this neither influences the fluorination procedure nor the isotopes composition.

The dried Ag_2S samples were fluorinated in nickel bombs under approximately 37 kPa of fluorine gas (F_2) at 250 °C overnight. The evolved SF_6 was purified cryogenically and then by a gas chromatography. Because of the small amount of samples (< 0.5 mg Ag_2S), a microvolume cold finger of an isotope ratio mass spectrometer (Thermo Scientific MAT 253) working in dual-inlet mode was used to concentrate the sample gas for isotope analysis [24]. The analytical uncertainty (1 σ) for the instrument was 0.25 ‰ for $\delta^{34}S$, 0.010 ‰ for $\Delta^{33}S$ and 0.062 ‰ for $\Delta^{36}S$ obtained by replicate analysis (N = 4) of IAEA-S-1 over a period of four weeks (one week per IAEA-S-1 analyzed) when the processed sulfate standards were also measured for sulfur isotopic composition.

RESULTS AND DISCUSSIONS

H_2S collection agents

The reduction product, H_2S, has to be collected and converted to Ag_2S before fluorination for isotope analysis. As mentioned above, $Cd(CH_3CO_2)_2$ [16, 17] and $AgNO_3$ [13]

have both been shown to be able to efficiently trap H_2S by forming CdS and Ag_2S precipitates, respectively. The CdS precipitate is further converted to Ag_2S by adding $AgNO_3$ solution [16, 17].

Conventional reducing solution commonly contains phosphorous acid (H_3PO_3) or hypophosphorous acid (H_3PO_2) [13], and phosphine (PH_3) is produced when the reducing solution is heated [18]. Once PH_3 gets in contact with $AgNO_3$, it reduces Ag^+ to Ag^0 and leads to excess precipitate in addition to Ag_2S [17]. To prevent this, Thode et al.[16] and Forrest et al.[17] used $Cd(CH_3CO_2)_2$ as the trapping solution. In particular, Forrest et al. [17] flushed the $Cd(CH_3CO_2)_2$ solution with N_2 for 15 minutes after CdS precipitates and prior to adding $AgNO_3$. This step was found to effectively remove PH_3 and thus no excess precipitate formed. However, Arnold et al. [13] found that when using $AgNO_3$ as the trapping solution, excess of Ag precipitate in the trap is not detrimental to the final isotope analysis of the sulfur content after fluorination. Because of this, $AgNO_3$ appears to be a better reagent to collect H_2S, given the environmentally toxic nature of Cd^{2+}.

In this study, we first employed 0.01 M $AgNO_3$ as the trapping solution. However, we observed spuriously high precipitate in the trap as soon as the reducing solution was heated, and the trapping solution turned to completely dark in a few minutes, even when there was no sulfate added to the reducing solution. At the same time, we noticed apparent silver mirror on the inside wall of the collection tube, indicating reduction of Ag^+ to Ag^0. This severely reduction of the $AgNO_3$ solution is probably due to the high production of PH_3 from our reducing solution. Different from the conventional reducing solution, our reducing solution used NaH_2PO_2 instead of H_3PO_2/H_3PO_3. NaH_2PO_2 starts to decompose and produce PH_3 at 90 °C, while H_3PO_3 effectively decomposes to yield

302 PH_3 at 200 °C. Therefore, at the temperature of the reduction experiment (i.e., 124 °C),
303 our reducing solution was presumably producing much more PH_3 than the conventional
304 reducing solution. To remove the excess precipitate other than Ag_2S caused by PH_3, we
305 used 1 M HNO_3, followed by 1 M NH_4OH to wash the precipitate formed in the $AgNO_3$
306 trapping solution. Only part of the precipitate was removed after these treatments and
307 there was still virtually more precipitate than expected. Thus, $AgNO_3$ is not a good choice
308 as the trapping solution, at least with regard to our reducing solution.

309 To avoid the reduction of Ag^+ by PH_3, we next tested 0.1 M $Cd(CH_3CO_2)_2$ as the
310 trapping solution and following the strategies described in Forrest et al.[17]. Despite this,
311 excess precipitation was still frequently observed after $AgNO_3$ was added to the trapping
312 solution for CdS to Ag_2S conversion. In particular, we noticed during the collection of
313 H_2S, yellow material was accumulated at the wall right above the surface of the
314 $Cd(CH_3CO_2)_2$ solution, indicating the formation of CdS. However, at the same time, the
315 entire $Cd(CH_3CO_2)_2$ solution became light brown and the brown color got deeper with
316 increasing trapping time. When $AgNO_3$ was added after the collection, the trapping
317 solution turned to dark with extensive precipitate at the same time. Obviously there were
318 still interferences between the trapping solution and/or $AgNO_3$ with the volatile
319 product(s) of the reducing solution. Similarly, excess precipitate remained after washing
320 with 1 M HNO_3 and 1 M NH_4OH. This, together with the toxic nature of Cd^{2+}, made us
321 decide to abandon $Cd(CH_3CO_2)_2$ as the trapping solution in our system.

322 Instead, we used 0.1 M NaOH as the trapping solution to collect H_2S. At this pH of
323 13, the trapped H_2S mainly existed in the form of HS^-, as shown in Figure 3a. Since the
324 NaOH trapping solution was purged with He, under this condition the dissolved O_2 was

very low and thus the trapped sulfide was stable. The use of NaOH as the trapping solution has two advantages, 1) the trapped H_2S can be precisely quantified in real-time using an ultraviolet spectrophotometer as described in section 2.4, and thus the progress toward to complete reduction of a sulfate sample can be monitored; 2) that no reaction between PH_3 and $AgNO_3$ occurs avoiding the production of excessive mass interference.

After sample collection, 1 mL 0.01 M $AgNO_3$ was added to the trap to produce Ag_2S. AgOH was produced at the same time, which changed to Ag_2O quickly. The suspension was allowed to settle down for 10-20 minutes after thoroughly shaking, and then a few drops of 68 % HNO_3 were added to acidify the trapping solution. Ag_2O was readily dissolved in the acidified solution and only Ag_2S remains.

H_2S yield

In the 0.1 M NaOH trapping solution, sulfide was mainly present in the form of HS^- (Figure 3a). Figure 3b shows the typical absorbance spectra of two Na_2S working standards (in 0.1 M NaOH matrix) and two NaOH trapping solution after 2 hours collection of H_2S, and as expected, the absorbance spectra peak ~ 230 nm, consistent with that from *Guenther et al.*[22]. In Figure 3c, an average of calibration curve over three days working standards is plotted.

As described in section 2.3, we had three different sulfate samples processed using our system, Na_2SO_4, $BaSO_4$-EB, and P-$BaSO_4$, and the time-resolved H_2S yields from these three materials are plotted in Figure 4. The real-time production of H_2S was monitored by ultraviolet determination of HS^- in the trapping solution every 15-20 minutes. Once the produced H_2S reached the amount expected from the starting sulfate, or no longer increases with time, the block heater was turned off and the reduction train

was flushed with He for 20 more minutes after the reaction tube cooled to room temperature.

In general, Na_2SO_4 was reduced faster than P-$BaSO_4$, and much faster than $BaSO_4$-EB. Regardless the quantity of the starting sulfate, after 1 hour of reduction, an average H_2S yield of (85.7 ± 10.3) % was reached when Na_2SO_4 was the starting material. In comparison, the H_2S yield after 1 hour of reduction was (63.9 ± 2.1) % for $BaSO_4$-EB and only (18.5 ± 0.04) % for P-$BaSO_4$. After 2 hours, a (99.5 ± 3.7) % yield was reached for Na_2SO_4, indicating the completion of the reduction. However, after 2 hours, it appeared that no more H_2S was produced for $BaSO_4$-EB and P-$BaSO_4$, and the yield remained at (80.4 ± 0.75) % for $BaSO_4$-EB and (28.5 ± 0.09) % for P-$BaSO_4$ after 4 or 5 hours. For two of the $BaSO_4$-EB samples, we let the reaction continue overnight, and the yield increased from 41.7 % and 34.5 % at 5 hours to 58.3 % and 86.5 %, respectively.

The final yields (yield after stopping the reaction) of Na_2SO_4, $BaSO_4$-EB and P-$BaSO_4$ sample with different quantities of sulfate are plotted in Figure 5. Overall, Na_2SO_4 was often 100 % reduced within two hours regardless the starting quantify, even when the drying agent and the cryogenic trap were put in-line, while a 100 % yield for $BaSO_4$-EB, and P-$BaSO_4$ was never observed even after overnight heating.

The different apparent reaction speeds and yields of H_2S between Na_2SO_4, $BaSO_4$-EB and P-$BaSO_4$ and the reducing solution likely reflect the effect of the sulfate salt solubility. Na_2SO_4 is soluble in water, while $BaSO_4$ has a very low solubility of 0.01 μmol/mL in water at 20 °C and less than 0.02 μmol/mL at ~120 °C [25]. Given the small volume of the reducing solution used (1 mL), there would be less than 2 % of the added $BaSO_4$ (if 1 μmol is added) dissolved. Our observations clearly point the sulfate ion

(SO_4^{2-}) or sulfuric acid (H_2SO_4) as the reactive species with the reducing acids, i.e. the sulfate salt has to be dissolved first in order to produce H_2S. This explains why the $BaSO_4$ samples reacted so slowly with the reducing solution relative to Na_2SO_4. In addition, if there is excess Ba^{2+} ion in the solution (due to dissolve of excess $BaCl_2$ that used to precipitate $BaSO_4$ from Na_2SO_4), it will inhibit the dissolution of $BaSO_4$ as the dissociation equilibrium of $BaSO_4$ will be pushed to the $BaSO_4$ side, following Le Chatelier's principle. This probably explains why the reducing reaction with P-$BaSO_4$ was faster than that with $BaSO_4$-EB. To confirm the effect of excess Ba^{2+} ion on reducing $BaSO_4$, we made a few $BaSO_4$ samples with extensively more Ba^{2+} by adding 1 mL 0.1 M $BaCl_2$ to 1 mL 1 mM Na_2SO_4 solution. The samples were then directly dried without removing supernatant from the precipitate. For these samples, after the reduction started, we measure the trapping solution every hour for 7 hours, and no H_2S was detected at all times.

Therefore, the solubility of the sulfate salt largely affects the reducing speed and the overall yield. We thus recommend to extract and convert sulfate in natural samples to Na_2SO_4 whenever possible when applying our reducing solution. The extraction of sulfate can be conducted using the IC method described in Geng et al. [26] or the anion-exchange resin method described in Le Gendre et al. [21]. In case of $BaSO_4$ is unavoidable, excess Ba^{2+} should be removed after precipitating $BaSO_4$ with $BaCl_2$, and increasing the volume of reducing solution (e.g., to 10 mL instead of 1 mL) and/or reaction time may improve the yield.

Isotope analysis of the standard materials

Since the overall goal of reducing sulfate to sulfide is to perform the four-sulfur isotope analysis, we processed three different barium sulfate standards, IAEA-SO-5, IAEA-SO-6 and NBS-127, which were equivalent to P-$BaSO_4$ samples. Unfortunately there are no international standards in sodium sulfate form and thus a strict comparison of the isotopic precision of the reduction step for the two chemical forms is impossible. Even a simple comparison of the salt from an identical sulfate batch is not possible as $BaSO_4$ reduction will never reach a full decomposition. The fluorination yield from Ag_2S to SF_6 and sulfur isotopic composition measured from these standards are listed in Table 1. The fluorination yield ranges from 84.6 % to 113.5 % and is with an average of (101 ± 7.5) %, except one standard with a low yield of 26.1 %. Regardless the fluorination yield, the measured isotopic values of all processed sulfate standards are statistically consistent with their accepted values, including the one with relatively low yield (26.1 %). The measured $\delta^{34}S$ (‰)$_{VCDT}$ values of all standards with different quantities of sulfur (0.34 - 2 μmol in SF_6) versus their accepted $\delta^{34}S$ (‰)$_{VCDT}$ values are plotted in Figure 6. A least-square linear regression gives a slope of (0.99 ± 0.01), suggesting a good reproducibility and the conservation of sulfur isotopic composition during the reduction of sulfate to sulfide using our reducing system, despite the reduction yields of these standard materials were not 100%. This is not a surprise. In fact, if any sulfur isotope fractionation occurs during the reduction, it would be between the solid $BaSO_4$ and the dissolved part HSO_4^- (the form of SO_4^{2-} in concentrated acid solution), but not in the step(s) from SO_4^{2-} to H_2S because the dissolved part is 100 % converted to H_2S. Kusakabe and Robinson [27] found that sulfur isotope fractionation between solid $BaSO_4$ and the dissolved HSO_4^- in the $BaSO_4$- HSO_4^--H_2O system is very small (less than 0.4 ‰ in the temperature range from

110 to 350 °C), which could explain why the solubility effect seems to not affect the isotopic measurements.

For these standards, we also reported the $\Delta^{33}S$ and they are all not distinct from what can be expected from mass-dependent fractionation. However, we did not include the $\Delta^{36}S$ values as when these standards were measured, the mass spectrometer had a high and variable background at m/z = 131 up to 50 mV which made the $\delta^{36}S$ values drifting and not reliable.

C**ONCLUSION**

We present a simple and reliable reducing method modified from the literature for the conversion of sulfate to sulfide for four-sulfur isotope analysis. This system is simple to set up, easy to replace and cheap to acquire and is made of sealed test tubes and PEEK flowing lines (metal part, e.g. needle, in contact with the hot reducing solution is not allowed). This method uses a reducing solution made of 100 mL 57 % HI and 13 g NaH_2PO_2, and a very small amount (1 mL) reducing solution was demonstrated to be able to completely reduce soluble sulfate salt (0.5 - 2.5 μmol) to sulfide within 2 hours, minimizing the use of relatively expensive HI acid. In practice, nothing prohibits the recycling of the used reducing solution by adding a few mgs of NaH_2PO_2 in order to reduce I_2 back to I^- in a boiling flask if the used solution turns brown [14]. In addition, the reduction train avoids distillation apparatus and multiple reduction trains can be operated at a time, making it easier to process multiple samples simultaneously. The use of NaOH as the trapping solution allows the assessment of reduction yield directly from UV determination of HS^- in the trapping solution.

This new approach was demonstrated to produce H_2S very rapidly with a 100 % recovery when soluble sulfate salt was used (e.g., Na_2SO_4), as opposed to $BaSO_4$ for which the kinetic was slow and conversion never reached 100 % even after overnight reaction. However, despite the relatively low reduction yield for $BaSO_4$, there was no significant isotope fractionation effect induced by the reduction. As it is the dissolved part of sulfate salt that reacts with the reducing solution, this method is best suitable for natural samples containing soluble sulfate (e.g., aerosol, snow and ice core), which can be extracted (e.g., by the resin method) and converted to Na_2SO_4. Barite precipitate method for sulfate extraction and purification is not recommended due to the inhibition of the salt solubility on the reduction speed and yield. In case of $BaSO_4$ is the main form of sulfate (e.g., barite), increasing the volume of the reducing solution and/or the reaction time may improve the H_2S yield with no guarantee of a complete conversion. While poor conversion and fluorination yields do not seem to introduce isotope fractionations, poor yield reduces the sensitivity of the method to sample sizes above few micromoles of sulfate and may also have consequence on the mass-dependent slopes between sulfur isotope ratios as $^{33}S/^{32}S$ ratio of the international standard have never been calibrated.

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References

[1] D. E. Canfield. Biogeochemistry of Sulfur Isotopes. *Reviews in Mineralogy and Geochemistry* **2001**, *43*, 607.

[2] J. Farquhar, H. M. Bao, M. Thiemens. Atmospheric influence of Earth's earliest sulfur cycle. *Science* **2000a**, *289*, 756.

[3] G. Feulner. The Faint Young Sun Problem. *Rev. Geophys.* **2012**, *50*.

[4] J. Farquhar, J. Savarino, T. L. Jackson, M. H. Thiemens. Evidence of atmospheric sulphur in the martian regolith from sulphur isotopes in meteorites. *Nature* **2000b**, *404*, 50.

[5] J. Cole-Dai, D. Ferris, A. Lanciki, J. Savarino, M. Baroni, M. H. Thiemens. Cold decade (AD 1810-1819) caused by Tambora (1815) and another (1809) stratospheric volcanic eruption. *Geophys. Res. Lett.* **2009**, *36*.

[6] J. Savarino, A. Romero, J. Cole-Dai, S. Bekki, M. H. Thiemens. UV induced mass-independent sulfur isotope fractionation in stratospheric volcanic sulfate. *Geophys. Res. Lett.* **2003**, *30*.

[7] M. Baroni, M. H. Thiemens, R. J. Delmas, J. Savarino. Mass-independent sulfur isotopic compositions in stratospheric volcanic eruptions. *Science* **2007**, *315*, 84.

[8] A. Lanciki, J. Cole-Dai, M. H. Thiemens, J. Savarino. Sulfur isotope evidence of little or no stratospheric impact by the 1783 Laki volcanic eruption. *Geophys. Res. Lett.* **2012**, *39*.

[9] N. Wu, J. Farquhar, H. Strauss, S.-T. Kim, D. E. Canfield. Evaluating the S-isotope fractionation associated with Phanerozoic pyrite burial. *Geochim Cosmochim Ac* **2010**, *74*, 2053.

[10] P. Sansjofre, P. Cartigny, R. I. F. Trindade, A. C. R. Nogueira, P. Agrinier, M. Ader. Multiple sulfur isotope evidence for massive oceanic sulfate depletion in the aftermath of Snowball Earth. *Nat Commun* **2016**, *7*.

[11] S. Ono, B. Wing, D. Rumble, J. Farquhar. High precision analysis of all four stable isotopes of sulfur (32S, 33S, 34S and 36S) at nanomole levels using a laser fluorination isotope-ratio-monitoring gas chromatography–mass spectrometry. *Chem Geol* **2006**, *225*, 30.

[12] Y. Ueno, S. Aoyama, Y. Endo, F. Matsu'ura, J. Foriel. Rapid quadruple sulfur isotope analysis at the sub-micromole level by a flash heating with CoF3. *Chem Geol* **2015**, *419*, 29.

[13] G. L. Arnold, B. Brunner, I. A. Müller, H. Røy. Modern applications for a total sulfur reduction distillation method - what's old is new again. *Geochem T* **2014**, *15*, 4.

[14] M. C. van Grondelle, F. van de Craats, J. D. van der Laarse. Microcoulometric determination of total inorganic sulphur in water by a hydroiodic acid reduction method. *Anal Chim Acta* **1977**, *92*, 267.

[15] K. Toshiyasu, T. Tomoo, Y. Yooko, K. Ikuko. Tin (II)-Strong Phosphoric Acid. A New Reagent for the Determination of Sulfate by Reduction to Hydrogen Sulfide. *B Chem Soc Jpn* **1955**, *28*, 641.

[16] H. G. Thode, J. Monster, H. B. Dunford. Sulphur isotope geochemistry. *Geochim Cosmochim Ac* **1961**, *25*, 159.

[17] J. Forrest, L. Newman. Silver-110 microgram sulfate analysis for the short time resolution of ambient levels of sulfur aerosol. *Anal. Chem.* **1977**, *49*, 1579.

[18] J. B. Davis, F. Lindstrom. Spectrophotometric microdetermination of sulfate. *Anal. Chem.* **1972**, *44*, 524.

[19] G. Norwitz. Spectrophotometric determination of sulphate in propellents and nitrocellulose. *Analyst* **1971**, *96*, 494.

[20] L. Gustafsson. Determination of ultramicro amounts of sulphate as methylene blue—I: The colour reaction. *Talanta* **1960**, *4*, 227.

[21] E. Le Gendre, E. Martin, B. Villemant, P. Cartigny, N. Assayag. A simple and reliable anion-exchange resin method for sulfate extraction and purification suitable for multiple O- and S-isotope measurements. *Rapid Commun. Mass Spectrom.* **2017**, *31*, 137.

[22] E. A. Guenther, K. S. Johnson, K. H. Coale. Direct Ultraviolet Spectrophotometric Determination of Total Sulfide and Iodide in Natural Waters. *Anal. Chem.* **2001**, *73*, 3481.

[23] R. J. Williams. Determination of inorganic anions by ion chromatography with ultraviolet absorbance detection. *Anal. Chem.* **1983**, *55*, 851.

[24] D. A. Yang, G. Landais, N. Assayag, D. Widory, P. Cartigny. Improved analysis of micro- and nanomole-scale sulfur multi-isotope compositions by gas source isotope ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* **2016**, *30*, 897.

[25] C. W. Blount. Barite solubilities and thermodynamic quantities up to 300 ^{o}C and 1400 bars. *Am Mineral* **1977**, 9.

[26] L. Geng, A. J. Schauer, S. A. Kunasek, E. D. Sofen, J. Erbland, J. Savarino, D. J. Allman, R. S. Sletten, B. Alexander. Analysis of oxygen-17 excess of nitrate and sulfate at sub-micromole levels using the pyrolysis method. *Rapid Commun. Mass Spectrom.* **2013**, *27*, 2411.

[27] M. Kusakabe, B. W. Robinson. Oxygen and sulfur isotope equilibria in the $BaSO_4$-HSO_4^--H_2O system from 110 to 350°C and applications. *Geochim Cosmochim Ac* **1977**, *41*, 1033.

[28] S. Halas, J. Szaran. Improved thermal decomposition of sulfates to SO_2 and mass spectrometric determination of $\delta^{34}S$ of IAEA SO-5, IAEA SO-6 and NBS-127 sulfate standards. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 1618.

Table 1. Fluorination yield and the measured isotopic values of the sulfate standards processed with the protocol described in this study.

Figure Legends:

Figure 1. A sketch of the reduction train. a: block heater and the reduction tube; b1 & b2: gas washing tubes; c: H_2S collection tube.

Figure 2. A sketch of the flow system containing multiple reaction trains. "T1...Tn" indicate the reduction trains assembled.

Figure 3. a. Percents of H_2S and HS^- in solutions with different pH calculated with pK_{a1} of 7.0 and pK_{a2} of 19, the vertical dashed line indicates the pH of the trapping solution used in this study; b. Absorbance spectra of Na_2S working standards and trapping solutions after 1 hour collection, the vertical dashed line indicates the absorbance peak of 230 nm; c. a three-day averaged calibration curve for H_2S quantification.

Figure 4. Time-resolved yields of H_2S from the reduction of dry Na_2SO_4, $BaSO_4$-EB ($BaSO_4$ with excess Ba^{2+}) and P-$BaSO_4$ (pure $BaSO_4$).

Figure 5. Yields of H_2S from the reduction of Na_2SO_4, $BaSO_4$-EB ($BaSO_4$ with excess Ba^{2+}) and P-$BaSO_4$ (pure $BaSO_4$) at different sulfate quantities at the time the reaction is stopped.

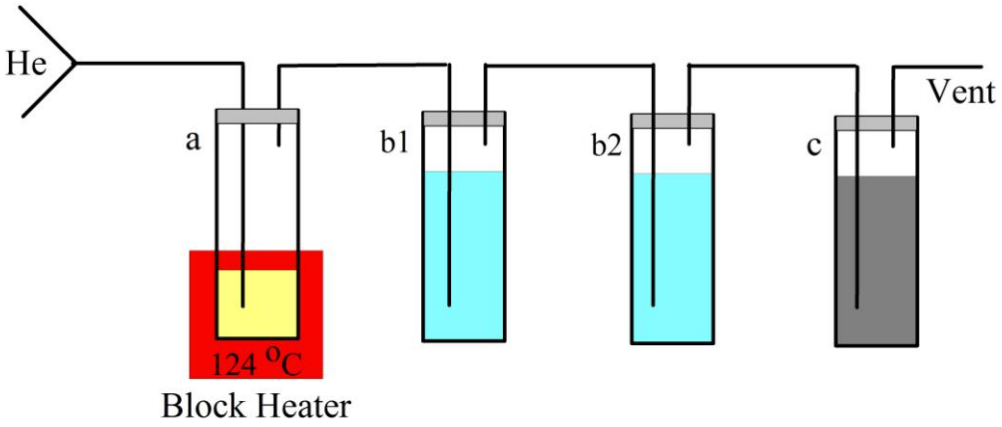
Figure 6. Measured versus accepted $\delta^{34}S$ $(‰)_{VCDT}$ values of IAEA-SO-5, IAEA-SO-6 and NBS-127. The reduction of these sulfate standards to H_2S were conducted using the protocol described in this study.

Table 1. Fluorination yield and measured isotopic ratios of the sulfate standards processed with this system. The values of $\Delta^{36}S$ are not reported as when the samples were measured the mass spectrometer had a high background of mass 131 (15 to 50 mV) and thus the $\Delta^{36}S$ data were discarded. The initial mass of the $BaSO_4$ standards were only approximately weighed, and the mass(es) in Ag_2S form were obtained according to the measured H_2S production after ~ 5 hours of reduction.

Standards	Ag_2S (mg)	SF_6 yield (%)	$\Delta^{33}S$ values vs CDT (‰)		$\delta^{34}S$ values vs CDT (‰)		Accepted $\delta^{34}S^a$ values vs CDT (‰)
NBS-127	0.20	101.7	0.015	0.025 ± 0.010	19.8	21.6 ± 1.3	20.3 ± 0.5
	0.10	105.3	0.018		22.4		
	0.08	93.7	0.033		22.8		
	0.12	98.2	0.034		21.4		
IAEA-SO-5	0.51	104.6	0.063	0.097 ± 0.071	0.7	0.7 ± 0.2	0.5 ± 0.5
	0.52	101.6	0.052		0.7		
	0.82	*26.1*	*0.203*		0.8		
	0.21	99.3	0.067		0.5		
IAEA-SO-6	0.41	113.5	0.077	0.086 ± 0.020	-34.0	-33.5 ± 0.6	-34.1 ± 0.5
	0.46	106.9	0.065		-33.9		
	0.13	102.0	0.110		-32.9		

	0.15	84.6	0.090		-32.9		

a. Accepted values are taken from Halas and Szaran [28].



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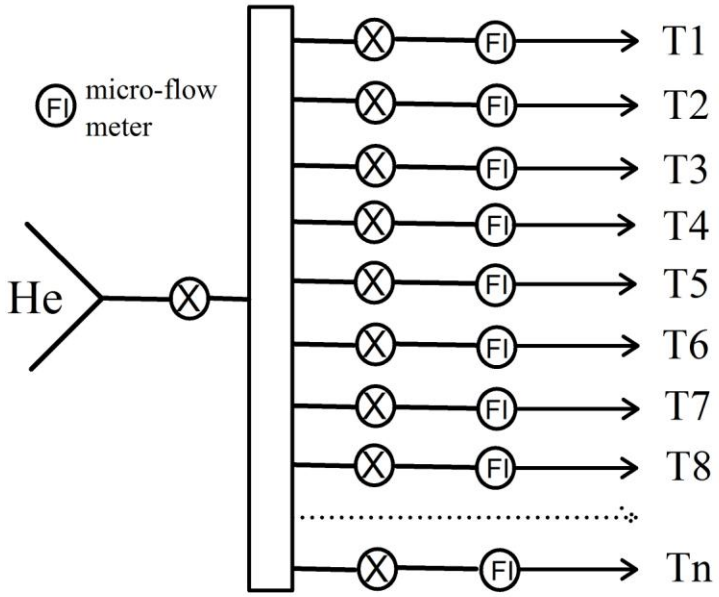

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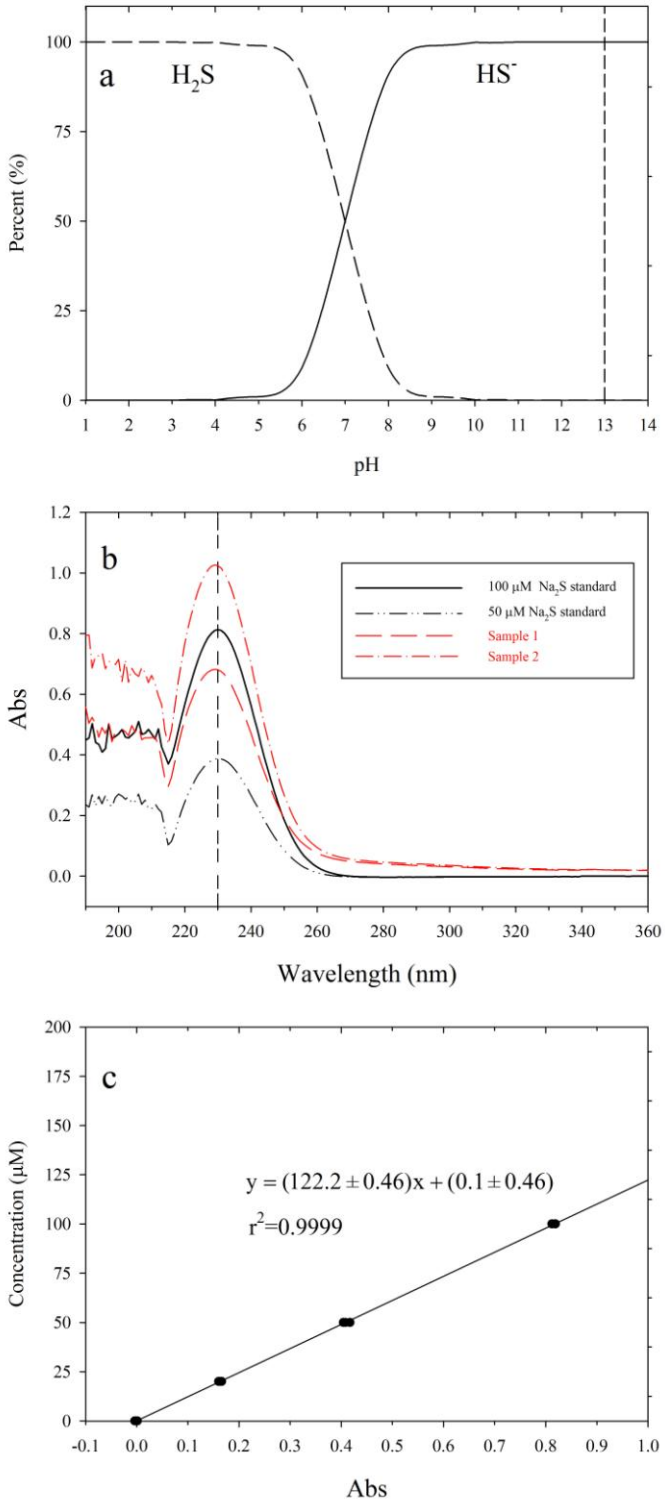

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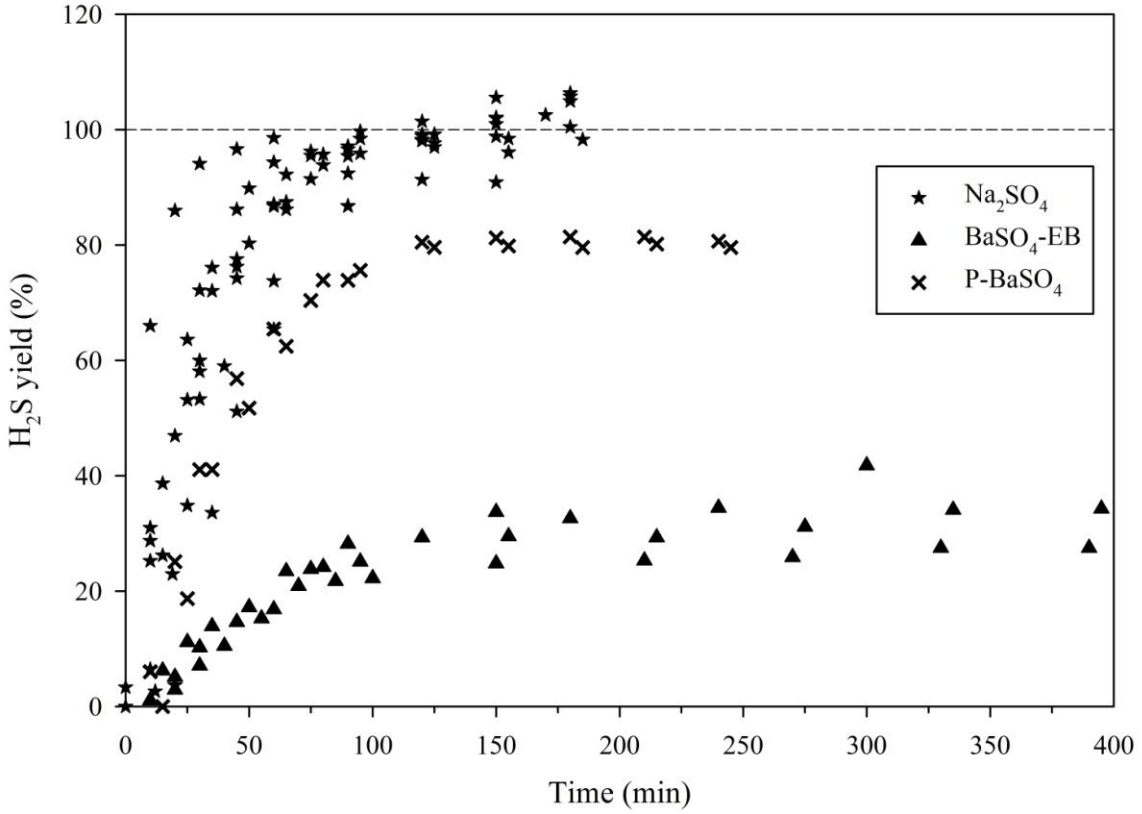

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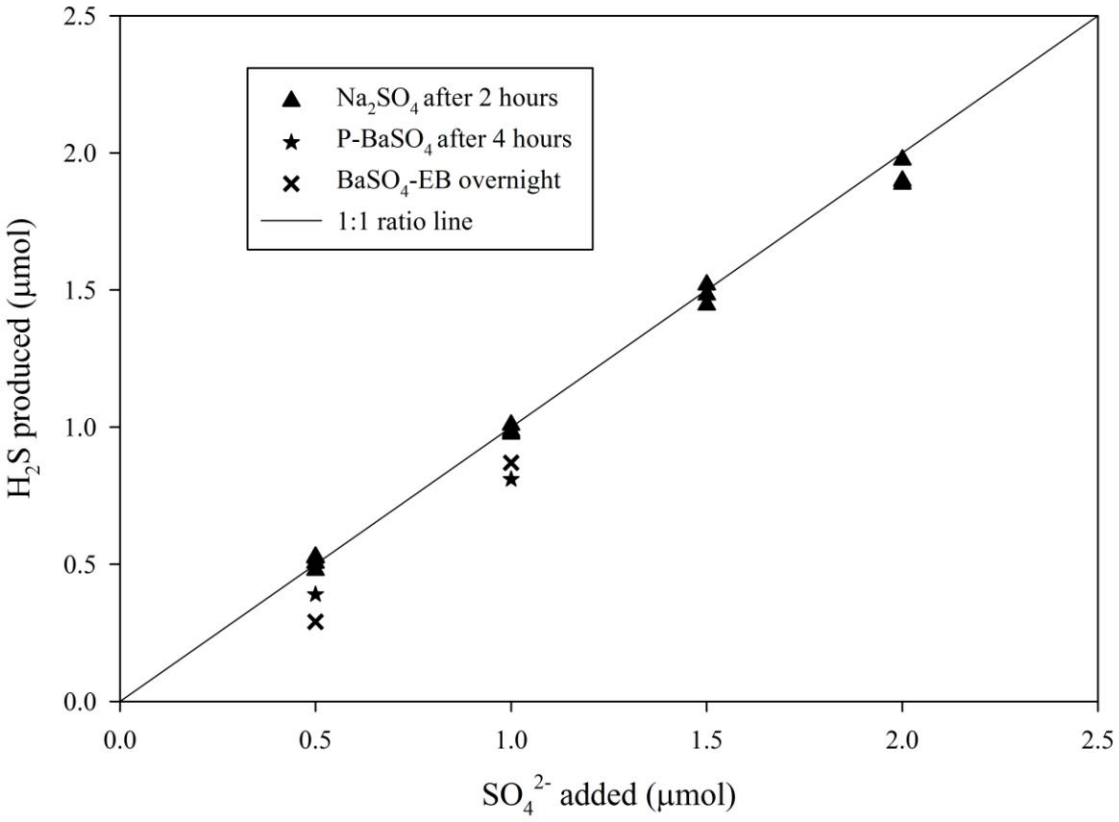

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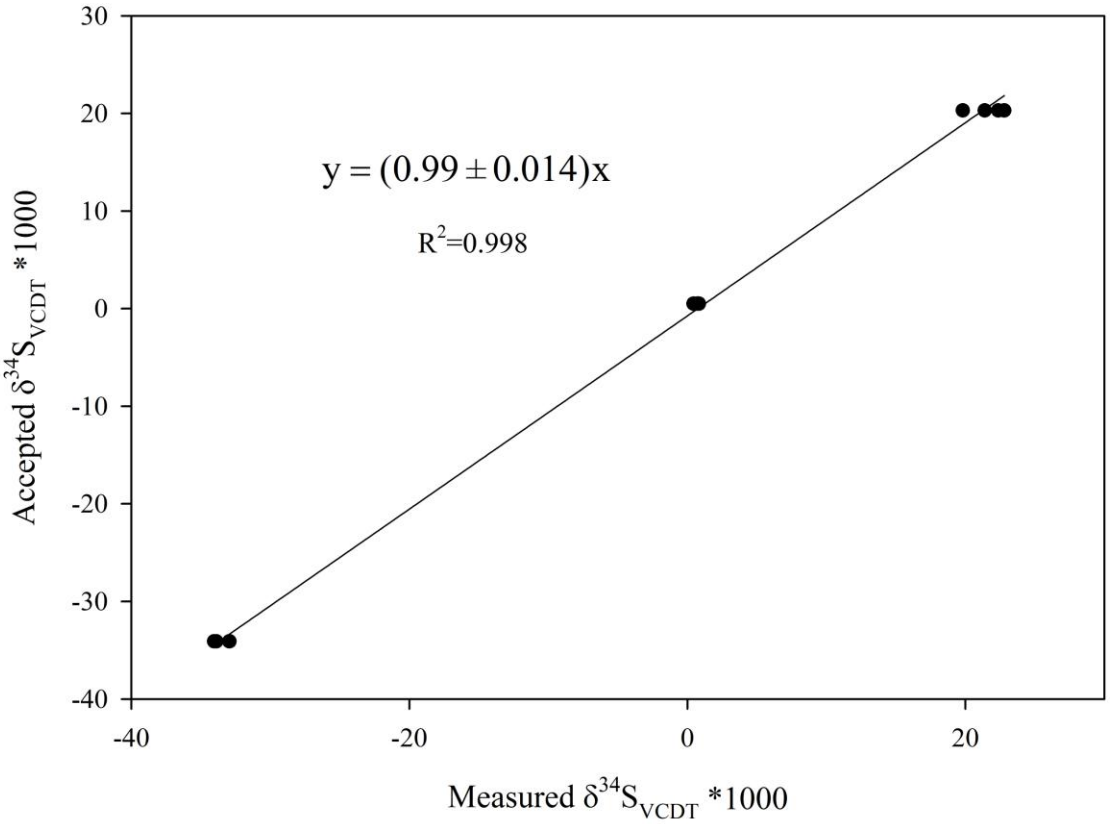

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