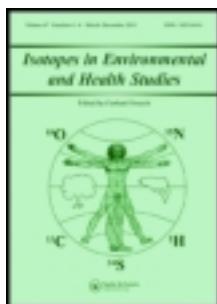


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## Comparison of sample preparation methods for stable isotope analysis of dissolved sulphate in forested watersheds

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Pretreatment methods for measuring stable sulphur ( $\delta^{34}\text{S}$ ) and oxygen ( $\delta^{18}\text{O}$ ) isotope ratios of dissolved sulphate from watersheds have evolved throughout the last few decades. The current study evaluated if there are differences in the measured stable S and O isotope values of dissolved sulphate from forested watersheds when pretreated using three different methods: Method 1 (M1): adsorb sulphate on anion exchange resins and send directly to isotope facility; Method 2 (M2): adsorb sulphate on anion exchange resins, extract sulphate from anion exchange resins, and send the produced  $\text{BaSO}_4$  to the isotope facility; and Method 3 (M3): directly precipitate  $\text{BaSO}_4$  without anion exchange resins with the precipitates being sent to the isotope facility. We found an excellent agreement of the  $\delta^{34}\text{S}_{\text{sulphate}}$  values among all the three methods. However, some differences were observed in the  $\delta^{18}\text{O}_{\text{sulphate}}$  values (M1 versus M2:  $-1.5\%$ ; M1 versus M3:  $-1.2\%$ ) associated with possible O contamination before isotope measurement. Several approaches are recommended to improve the pretreatment procedures for  $\delta^{18}\text{O}_{\text{sulphate}}$  analysis.

**Keywords:** isotope measurements; methods and equipment; isotope ratio mass spectrometry; natural abundances; oxygen-18; sample preparation methods; sulphate; sulphur-34; watersheds

### 1. Introduction

The use of stable sulphur (S) isotope values of sulphate ( $\delta^{34}\text{S}_{\text{sulphate}}$ ) has provided important information on S biogeochemistry, including the evaluation of the contribution of atmospheric S inputs to the acidification of both aquatic and terrestrial ecosystems [1–3]. Some isotope studies of the S cycle have also determined oxygen (O) isotope ratios of sulphate ( $\delta^{18}\text{O}_{\text{sulphate}}$ ) for ascertaining the relative roles of different sulphate sources and transformations [4–6]. Generally, two approaches using the isotopic composition of sulphate have been applied: tracer experiments in which sulphate with a known and distinctive isotopic composition has been added or S biogeochemistry studies exploiting the patterns of the natural abundance of S and O isotope ratios in aquatic and terrestrial ecosystems [2]. The latter approach has been used extensively for understanding the S biogeochemistry as part of the Hubbard Brook Ecosystem Study in the White

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Mountain National Forest in New Hampshire, USA. These studies have included evaluating the role of atmospheric deposition [7], historical changes in S biogeochemistry [8], the role of mineral weathering S inputs [9,10], simulation modelling of S biogeochemistry [11], and the overall evaluation of S budgets in forested watersheds [3,8,12,13].

To assure the comparability of results of stable isotope measurements, it is important to provide information about procedures that were used to prepare and analyse dissolved sulphate samples for isotopic determinations since procedures often vary between laboratories [14]. In many earlier studies, barium (as BaCl<sub>2</sub> solution) was added in excess to convert dissolved sulphate in water samples into BaSO<sub>4</sub> precipitates, followed by a removal of these precipitates from the water samples by filtration [4,7,8,15]. Some studies have suggested that there may be interferences associated with this method, due to the co-precipitation of other solutes: for example, dissolved organic matter (DOM) [16,17] and nitrate [18]. The O contained in DOM may severely compromise the accurate determination of O isotope ratios of sulphate in the BaSO<sub>4</sub> precipitates. In Standard Methods [16], organic material is listed as one of the major interferences for the turbidimetric method for the examination of sulphate concentrations (4500-SO<sub>2</sub><sup>2-</sup> E), because large amounts of organic material may result in incomplete BaSO<sub>4</sub> precipitation from the solution. Ristow *et al.* [17] also suggested that the BaSO<sub>4</sub> turbidimetric method is problematic for solutions with high concentrations of organic material. Incomplete precipitation of sulphate may only result in very minor isotopic discrimination during mineral precipitation [18]. Michalski *et al.* [19] suggested that co-precipitation of nitrate with BaSO<sub>4</sub> in solutions with a nitrate-to-sulphate molar ratio greater than 2 occurs, with the co-precipitated nitrate potentially resulting in O isotope ratio measurements that are not accurately reflecting the  $\delta^{18}\text{O}$  value of the dissolved sulphate.

Since the 1990s, anion exchange resins have been increasingly used for the collection of dissolved sulphate from water samples [5,6,20–22]. This approach has been widely accepted [23], because it offers several advantages. It avoids problems due to incomplete precipitation of BaSO<sub>4</sub>, especially for water samples with low sulphate concentrations. It also eliminates the inconvenience of transporting large volumes of water to the laboratory for sample preparation and analysis [24].

The current study provides new results on the impact of sample handling procedures on measured isotope ratios, based on selected aquatic samples from forested watersheds, since examining possible experimental procedures is of critical importance prior to adapting the procedures for collecting isotopic data. This study was designed to evaluate whether there are differences in the measured stable S and O isotopic composition of dissolved sulphate from freshwaters when extracted using several different methods. Therefore, the objective of our study was to test the influence of these different pretreatment and processing techniques for obtaining BaSO<sub>4</sub> on the accuracy of  $\delta^{34}\text{S}$  and  $\delta^{18}\text{O}$  values obtained for dissolved sulphate from a variety of different water samples in forested watersheds.

## 2. Materials and methods

Our study was designed to compare three methods used for preparing dissolved sulphate samples for stable isotope analysis, as follows (Figure 1):

- (1) Method 1 (M1): Percolating sulphate-containing solutions through ion exchange resins at the research site and sending the sulphate-containing anion exchange resins to an isotope laboratory for further processing.
- (2) Method 2 (M2): Percolating sulphate-containing solutions through ion exchange resins, eluting the sulphate from the columns at the research site, and sending the extracted dissolved sulphate from the resins to an isotope laboratory for analyses.

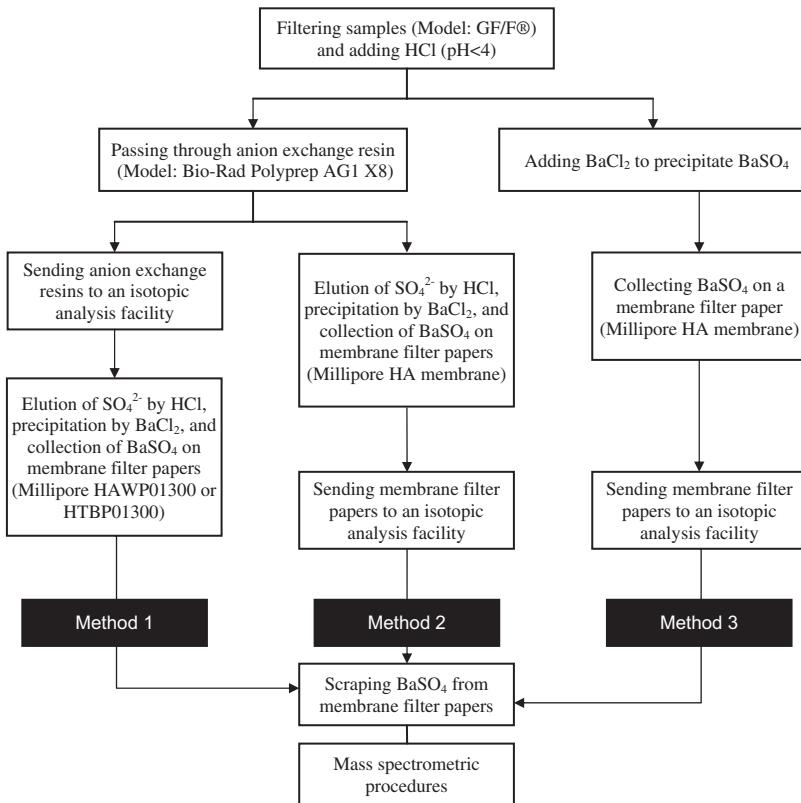


Figure 1. Flow diagram of the three methods used for sample preparation of sulphate from aqueous samples for sulphur and oxygen isotope ratio measurements.

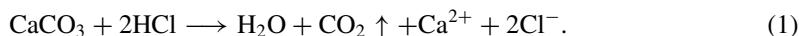
(3) Method 3 (M3): Directly precipitating  $\text{BaSO}_4$  from sulphate-containing solutions via the addition of  $\text{BaCl}_2$  solution at the research site and submitting the filtered, washed, and dried  $\text{BaSO}_4$  precipitate to an isotope laboratory for analyses.

## 2.1. Sampling and chemical analysis

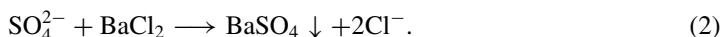
Water samples were collected from the Arbutus Lake Watershed (ALW) in the Adirondack Park in New York State, USA, and from the Hubbard Brook Experimental Forest (HBEF) in the White Mountains of the State of New Hampshire, USA. For the comparison between M1 and M2, we analysed samples from five stream sites (wetland, S14, S15, lake inlet, and lake outlet) taken in May and August 2008 and from four lake sampling locations (surface and bottom waters of upper (L1) and lower (L2) sites, respectively) obtained in August 2008 in ALW. At the HBEF, we sampled streams at seven locations (W1, W3, W5, W6, W9, Paradise Brook (PB), and Bear Brook (BB)) in July 2008. In November 2008, we obtained additional samples at ALW (S14, S15, lake inlet, and lake outlet). Further details on sampling site information at ALW are provided in [25]; information on sampling sites at HBEF is described in [10,13]. Field samples were transported on ice to the laboratory within 1 or 2 days and then stored in the dark at  $4^\circ\text{C}$ . Further sample processing was completed within 1 week. Sulphate and nitrate solute concentrations were measured using Dionex ion chromatography. Dissolved organic carbon (DOC) concentrations were determined using a Tekmar-Dohrmann Phoenix 8000 TOC analyser.

## 2.2. Sample preparation procedures for isotope analyses

Water samples of 1 l were filtered using a GF/F filter (Whatman®). All samples for isotope measurements were analysed in triplicates. After filtration, 1 ml of 3 molar HCl was added to the filtrate to remove  $\text{HCO}_3^-$  in order to prevent co-precipitation of  $\text{BaCO}_3$  (Figure 1 and Equation (1)):



M1: The filtrates were passed through anion exchange resin columns (Bio-Rad Polyprep, AG 1X-8, Bio-Rad Laboratories, Hercules, CA) to retain the sulphate [10]. Ion chromatographic analysis of sulphate in the effluent confirmed that all sulphate had been retained in the resins. The resins with the adsorbed sulphate were shipped immediately to the Isotope Science Laboratory (ISL) at the University of Calgary, Alberta, Canada, for isotope analyses. At the ISL, sulphate was eluted from each column by adding three times 5 ml (in total 15 ml) of 3 molar HCl. Approximately 5 ml of a 0.5 molar  $\text{BaCl}_2$  solution was added to quantitatively precipitate  $\text{BaSO}_4$ :



The  $\text{BaSO}_4$  precipitate was filtered using Millipore HAWP01300 (nominal pore size: 0.45  $\mu\text{m}$ , material: mixed cellulose ester) or Millipore HTBP01300 (nominal pore size: 0.45  $\mu\text{m}$ , material: polycarbonate) filters and washed thoroughly several times with deionised water to remove any residual chloride. The  $\text{BaSO}_4$  precipitate was subsequently recovered from the surface of the filter paper, washed with deionised water, air-dried, weighed, and stored.

M2: Filtration and passing of the water samples through anion exchange resin columns were performed in the Biogeochemistry Laboratory at the SUNY-ESF. The effluent sulphate was analysed by ion chromatography in order to confirm the resin capability of retaining all the sample sulphate. The sulphate on the anion exchange resin columns was eluted and precipitated as  $\text{BaSO}_4$ , as described above. The  $\text{BaSO}_4$  precipitate was collected on Millipore HA membrane filters (pore size: 0.45  $\mu\text{m}$ , material: mixed cellulose ester), air-dried, and weighed. The membrane filters containing the filtered  $\text{BaSO}_4$  were sent to the ISL, where  $\text{BaSO}_4$  was recovered from the filters and the S and O isotope ratios were determined.

M3: One millilitre of 0.5 molar  $\text{BaCl}_2$  solution was added and stirred into 1 l of the filtered water sample to precipitate  $\text{BaSO}_4$ , and more  $\text{BaCl}_2$  was added to the samples with high sulphate concentrations. The precipitate was recovered by filtration using Millipore HA membrane filters (nominal pore size: 0.45  $\mu\text{m}$ , material: mixed cellulose ester), washed with deionised water, and air-dried. The membrane filters containing the filtered  $\text{BaSO}_4$  were sent to the ISL for isotope analyses. At the ISL,  $\text{BaSO}_4$  was removed from the membrane filters for subsequent isotope analyses.

## 2.3. Internal laboratory standards

Along with sample measurements, we analysed two internal laboratory standards (Standards A and B) in order to compare the stable S and O isotope ratios for the three methods. Standard A (Std A) was produced using manufactured sulphuric acid ( $\text{H}_2\text{SO}_4$ ) from Baker Analyzed ACS reagent and Standard B (Std B) was obtained from VWR. The concentrations and the stable S and O isotope ratios of the standards are reported in Section 3.

## 2.4. Mass spectrometric procedures

Using solid  $\text{BaSO}_4$  material from each method, sulphur dioxide ( $\text{SO}_2$ ) for mass spectrometric analyses was generated by thermal decomposition in an elemental analyser. Sulphur isotope ratios

were determined by continuous flow isotope ratio mass spectrometry (CF-IRMS) [26,27]. For O isotope analyses on sulphate, BaSO<sub>4</sub> oxygen was converted to CO at 1450 °C in a pyrolysis reactor (Finnigan TC/EA, Thermo Electron, Bremen, Germany). The resultant gas was subsequently swept with a He stream into a mass spectrometer (Finnigan MAT delta plus XL) for isotope ratio determinations in a continuous-flow mode (CF-IRMS) [5,28]. All isotope ratios are expressed in the conventional ‘delta ( $\delta$ ) notation’ in per mil (‰):

$$\delta(\text{‰}) = \left[ \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 10^3, \quad (3)$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the  $^{34}\text{S}/^{32}\text{S}$  or  $^{18}\text{O}/^{16}\text{O}$  ratios of the sample and the standard, respectively. The internationally accepted standards are Vienna-Canyon Diablo Troilite for S isotope ratios and Vienna Standard Mean Ocean Water for O isotope ratios. The analytical precision determined by long-term monitoring of international and internal laboratory standards and reference materials was  $\pm 0.3$  ‰ for the  $\delta^{34}\text{S}$  values and  $\pm 0.5$  ‰ for the  $\delta^{18}\text{O}$  values of sulphate.

### 2.5. Statistical evaluations

We tested for differences in the mean  $\delta^{34}\text{S}_{\text{sulphate}}$  and  $\delta^{18}\text{O}_{\text{sulphate}}$  values between M1 and M2 using PROC MIXED and accounted for the random block effect due to data and site individually (SAS, 1994). The difference in the adjusted least squares means (LSMEANS) between the methods was evaluated using Tukey’s means comparison test at  $\alpha = 0.05$  level using the following model:

$$Y_{ijk} = \mu + d_i + s_j + m_k + e_{ijk},$$

where  $Y_{ijk}$  is the observed stable isotope value,  $\mu$  the overall mean observation,  $d_i$  the random block effect of dates ( $i = 1-5$ ),  $s_j$  the random block effect of sites ( $j = 1-17$ ),  $m_k$  the fixed effect of methods ( $k = 1-2$ ), and  $e_{ijk}$  the random error terms.

Also, a linear regression of either  $\delta^{34}\text{S}_{\text{sulphate}}$  or  $\delta^{18}\text{O}_{\text{sulphate}}$  values between the methods was analysed to evaluate the slope of a regression line relating M1 to M2 using SigmaPlot (Version 11.0, Systat Software, Inc.).

For the comparison among the three methods (M1, M2, and M3), we used PROC GLM and accounted for the random block effect in the sites using SAS (Statistical Analysis Software for Microcomputers, SAS Institute Inc., 1994). LSMEAN among the methods was evaluated using Tukey’s means comparison test at  $\alpha = 0.05$  level using the following model:

$$Y_{ij} = \mu + s_i + m_j + e_{ij},$$

where  $Y_{ij}$  is the observed stable isotope value,  $\mu$  the overall mean observation,  $s_i$  the random block effect of sites ( $i = 1-5$   $\delta^{18}\text{O}$  or  $6$   $\delta^{34}\text{S}$ ),  $m_j$  the fixed effect of methods ( $j = 1-3$ ), and  $e_{ij}$  the random error terms.

Also, a one-way ANOVA was performed to test the differences among the methods of each site at  $\alpha = 0.05$  level using SAS.

## 3. Results

The results for the experiment on comparison of the inter-laboratory effects using anion exchange resins (M1 versus M2) are summarised in Table 1. The standard deviations of the three replicate measurements for each sample were in most cases within  $\pm 0.3$  ‰ for  $\delta^{34}\text{S}$  and  $\pm 0.5$  ‰ for  $\delta^{18}\text{O}$ , indicating excellent external reproducibility. For S isotope ratios of sulphate (Table 1), there was

no significant difference ( $F_{1,111} = 2.75$ ,  $p = 0.10$ ) between the  $\delta^{34}\text{S}_{\text{sulphate}}$  values measured using M1 (LSMEAN =  $3.8 \pm 0.3\text{‰}$ ) and M2 (LSMEAN =  $3.7 \pm 0.3\text{‰}$ ). The difference between M1 and M2 was  $0.1 (\pm 0.3 \text{ SD}) \text{‰}$ . Also the slope of the regression line relating M1 to M2 was 0.97 (Figure 2), indicating that there was no significant difference between the  $\delta^{34}\text{S}_{\text{sulphate}}$  values generated using M1 and M2, respectively. The  $\delta^{18}\text{O}_{\text{sulphate}}$  values, however, obtained with these two methods were significantly different ( $F_{1,110} = 137.18$ ,  $p < 0.01$ ) (Table 1), and M1 (LSMEAN  $2.1 \pm 0.3\text{‰}$ ) produced lower values than M2 ( $3.1 \pm 0.3\text{‰}$ ). The mean difference between M1 and M2 was  $-1.1 (\pm 0.7 \text{ SD}) \text{‰}$  and it ranged from  $-3.0$  to  $-0.4\text{‰}$ . Also, the slope and the intercept of the regression line comparing M1 to M2 were 0.78 and  $+1.5\text{‰}$ , respectively, showing a discrepancy of results between M1 and M2 (Figure 2).

Table 1. Mean solute sulphate concentrations and  $\delta^{34}\text{S}$  and  $\delta^{18}\text{O}$  values of M1 and M2 (standard deviation,  $n = 3$ ).

Location	2008 Month	Site	$\text{SO}_4^{2-}$ ( $\mu\text{mol/l}$ )	$\delta^{34}\text{S}$ (‰)		$\delta^{18}\text{O}$ (‰)	
				M1	M2	M1	M2
ALW	May	Inlet	56	4.2 (0.0)	4.0 (0.1)	2.1 (0.6)	5.1 (1.2)
		Outlet	41	3.7 (0.2)	3.5 (0.3)	2.2 (0.1)	3.5 (0.2)
		S14	75	1.1 (0.1)	0.8 (0.6)	0.0 (0.1)	0.9 (0.4)
		S15	94	3.9 (0.0)	3.7 (0.2)	1.6 (0.1)	3.2 (0.3)
		Wetland	56	4.2 (0.0)	4.0 (0.6)	2.0 (0.4)	4.8 (1.0)
	August	Inlet	40	4.4 (0.1)	4.2 (0.1)	2.1 (0.3)	2.7 (0.1)
		Inlet	44	4.6 (0.0)	4.6 (0.0)	1.3 (0.5)	2.6 (0.1)
		Outlet	43	4.6 (0.1)	4.6 (0.1)	2.5 (0.3)	3.0 (0.3)
		L1-0 m	43	4.6 (0.1)	4.6 (0.0)	1.9 (0.2)	3.1 (0.1)
		L1-6 m	40	4.8 (0.0)	4.9 (0.0)	2.5 (0.1)	2.9 (0.1)
		L2-0 m	42	5.4 (1.0)	4.6 (0.1)	2.3 (0.1)	2.7 (0.3)
		L2-4 m	41	4.4 (0.0)	4.6 (0.0)	1.9 (0.1)	2.7 (0.4)
		S14	64	2.0 (0.1)	2.2 (0.1)	0.1 (0.5)	1.1 (0.2)
		S15	56	3.8 (0.1)	4.0 (0.0)	1.4 (0.0)	2.2 (0.7)
		Wetland	44	4.3 (0.4)	4.6 (0.0)	0.9 (0.7)	2.7 (0.3)
HBEF	July	W1	41	3.4 (0.1)	3.4 (0.4)	2.0 (0.2)	3.3 (0.3)
		W3	37	3.9 (0.3)	3.7 (0.5)	2.6 (0.3)	3.8 (0.3)
		W5	42	2.0 (0.1)	2.2 (0.3)	3.8 (0.3)	4.2 (0.3)
		W6	36	3.4 (0.0)	3.4 (0.3)	2.4 (0.4)	3.1 (0.2)
		W9	33	4.4 (0.1)	4.0 (0.2)	2.7 (0.0)	3.2 (0.2)
		PB	35	3.2 (0.0)	2.9 (0.1)	2.9 (0.2)	3.5 (0.3)
		BB	38	1.6 (0.1)	1.5 (0.1)	2.8 (0.2)	3.5 (0.4)

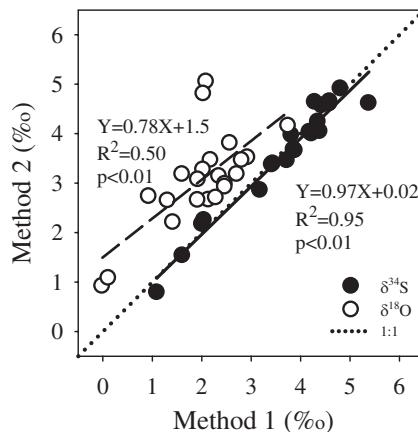


Figure 2. Comparisons of sulphur isotope ratios expressed as  $\delta^{34}\text{S}$  (closed circles) and oxygen isotope ratios expressed as  $\delta^{18}\text{O}$  (open circles) of sulphate between M1 and M2 ( $n = 22$ ). The regression line (dashed for oxygen; solid for sulphur) for each method is provided with a 1:1 relationship indicated with a dotted line.

The results for the experiment on comparison of the water-chemistry effects (e.g. nitrate/sulphate and DOC/sulphate) between the methods are summarised in Figure 3. At sampling sites with high molar ratios (10:11) of DOC to sulphate (e.g. inlet and outlet), insufficient precipitation of  $\text{BaSO}_4$  in M2 and M3 at the outlet site and impure precipitation of  $\text{BaSO}_4$  in M3 at the inlet and outlet sites were observed, compromising the isotope measurement comparisons (Figure 3). For the inlet and outlet samples, the precipitate of the eluents from the anion exchange resin in M2 was white, the expected colour of  $\text{BaSO}_4$  precipitate. In contrast, the precipitates generated using M3 were dark brown, suggesting co-precipitation of substantial amounts of DOC along with  $\text{BaSO}_4$ . For samples with molar ratios of DOC to sulphate less than 2 using M3, the precipitate was white suggesting no marked co-precipitation of DOC. Overall, there was no significant difference among the three methods for the  $\delta^{34}\text{S}_{\text{sulphate}}$  values ( $F_{2,53} = 0.18$ ,  $p = 0.83$ ), given the analytical precision of  $\delta^{34}\text{S}$  ( $\pm 0.3\text{‰}$ ), since LSMEANs for M1, M2, and M3 were 2.8, 2.9, and 2.7‰, respectively. In addition, the  $\delta^{34}\text{S}_{\text{sulphate}}$  values obtained from all the three methods were in agreement usually within 0.6‰. Despite the good agreement of the  $\delta^{34}\text{S}_{\text{sulphate}}$  values

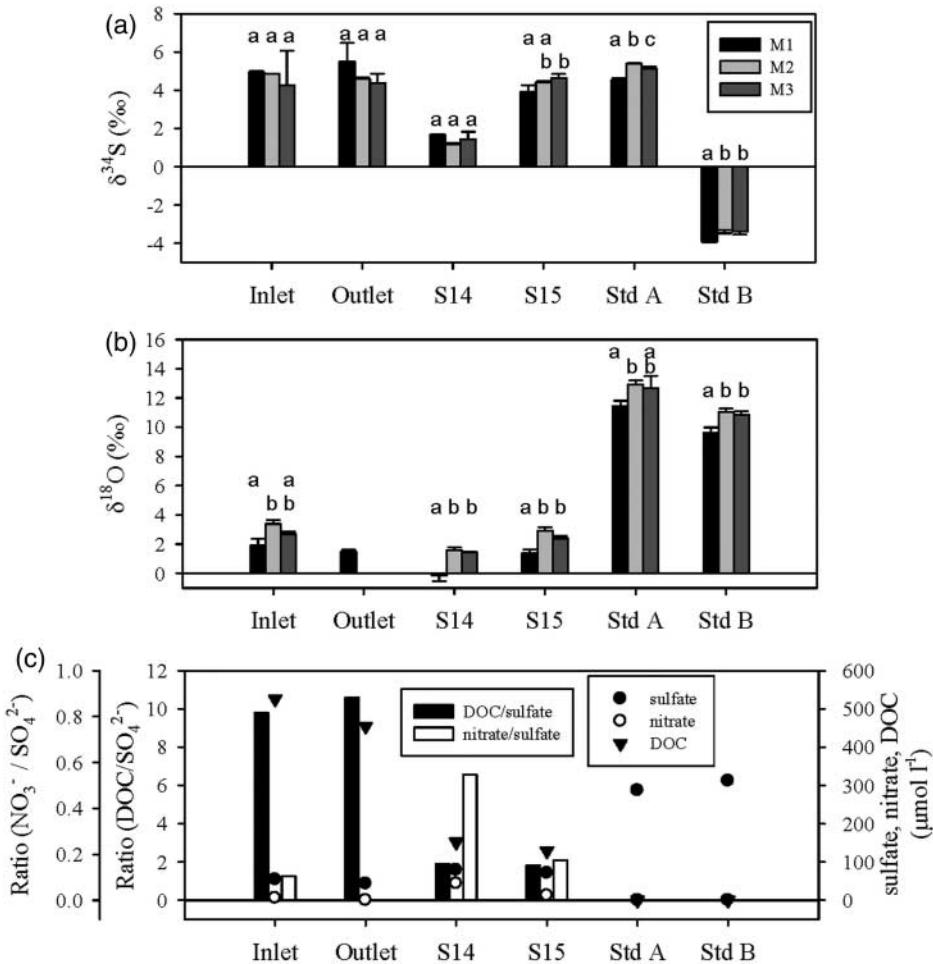


Figure 3. The  $\delta^{34}\text{S}$  and  $\delta^{18}\text{O}$  values of sulphate (panels A and B), concentrations of sulphate, nitrate, and DOC, and the molar ratios of both nitrate to sulphate and DOC to sulphate (panel C) at four sampling sites in the ALW in November 2008 and two internal laboratory standards (Std A and Std B) for the comparison among M1, M2, and M3. In panels A and B, letters above the bars indicate significant ( $p < 0.05$ ) differences among means. Error bars are standard errors.

among the methods, a mean difference between M1 and M2/M3 for each internal laboratory standard (Std A and Std B) was observed as shown in Figure 3. Although there was a small, but statistically significant difference between M2 and M3 in  $\delta^{34}\text{S}_{\text{sulphate}}$  of Std A (Figure 3), this difference (M2–M3 = 0.29‰) was within the analytical uncertainty of  $\pm 0.3$ ‰.

The  $\delta^{18}\text{O}_{\text{sulphate}}$  values excluding those of the outlet site showed, however, significant differences among the three methods ( $F_{2,44} = 83.87$ ,  $p < 0.01$ ). The highest LSMEAN of the  $\delta^{18}\text{O}_{\text{sulphate}}$  value was observed for M2 with 6.4‰, followed by M3 with 6.0‰ and M1 with 4.8‰. For the  $\delta^{18}\text{O}_{\text{sulphate}}$  values, the difference between M2 and M3 was 0.4‰, which is identical within the analytical uncertainty of  $\pm 0.5$ ‰. However, M1 resulted in  $\delta^{18}\text{O}_{\text{sulphate}}$  values that were 1.5 and 1.2‰ lower than those generated in M2 and M3, respectively. Potential reasons for the observed differences are discussed below.

#### 4. Discussion

The  $\delta^{34}\text{S}_{\text{sulphate}}$  values measured using the three different sample preparation methods showed no statistical differences. This result suggests that the use of different sample preparation procedures used in previous studies, for example, at HBEF [3,6–8,10–12], does not affect the accuracy and comparability of the reported  $\delta^{34}\text{S}$  values.

Significant differences in the  $\delta^{18}\text{O}_{\text{sulphate}}$  values between M1 and M2, however, were found, with M1 producing  $\delta^{18}\text{O}_{\text{sulphate}}$  values approximately 1.5‰ lower than those generated in M2. Considering the analytical precision for the  $\delta^{18}\text{O}_{\text{sulphate}}$  values of  $\pm 0.5$ ‰, M1 was statistically different from M2 and M3, with the latter two being statistically similar. The differences in the  $\delta^{18}\text{O}_{\text{sulphate}}$  values between M1 and M2/M3 suggest that sample processing may introduce artefacts in the measured O isotope ratios of sulphate. The role of sample processing might have been affected by two different factors: (1) an input from an unidentified O source with a  $\delta^{18}\text{O}_{\text{sulphate}}$  value different from that of the sulphate sample or (2) isotope effects during incomplete processing or precipitation of sulphate (Figure 1). Potential reasons for differences in the  $\delta^{18}\text{O}_{\text{sulphate}}$  values generated in M1 and M2/M3 are described below.

##### 4.1. DOM (DOC) as a source of bias in the reported $\delta^{18}\text{O}$ values of sulphate

DOM has been known to interfere with the precipitation of  $\text{BaSO}_4$  [16], potentially resulting in low sample recovery and precipitate contamination by DOM. Both these factors could affect the resultant  $\delta^{18}\text{O}_{\text{sulphate}}$  values obtained using M3. In the case of  $\delta^{34}\text{S}_{\text{sulphate}}$ , Priezel and Mayer [18] showed, however, that incomplete precipitation does not affect S isotope values significantly during precipitate formation. This explanation is consistent with the good agreement of our  $\delta^{34}\text{S}_{\text{sulphate}}$  values determined by the different preparation procedures. Oxygen in DOM generally ranges between 23 and 45 % by mass [29]. The  $\delta^{18}\text{O}$  values in DOM at the Harp Lake catchment in Canada ranged from 8.2 to 14.4‰ [30]. For the comparison of the  $\delta^{18}\text{O}_{\text{sulphate}}$  values from our study with those from previous studies, the only information we currently have on the  $\delta^{18}\text{O}_{\text{sulphate}}$  values of sulphate in precipitation and surface water at Hubbard Brook was provided by Miles *et al.* [22], who found mean precipitation and stream water  $\delta^{18}\text{O}_{\text{sulphate}}$  values of 8.0 and 3.4‰, respectively. Considering the analytical precision of  $\delta^{18}\text{O}$  of sulphate with  $\pm 0.5$ ‰, the observed difference of 0.4‰ between M2 (LSMEAN 6.4‰) and M3 (LSMEAN 6.0‰) is within the range of expected analytical variation. In addition, the observed analytical differences in the  $\delta^{18}\text{O}_{\text{sulphate}}$  values between M2 that used ion exchange resins and M3 using direct precipitations were much less than the differences in values typically associated with making biogeochemical inferences with respect to sulphate sources and S transformations (e.g.  $\gg 1$ ‰).

Therefore, we conclude that the inclusion of DOM oxygen in the BaSO<sub>4</sub> precipitates in M3 did not cause substantial differences in the obtained  $\delta^{18}\text{O}_{\text{sulphate}}$  values compared with those obtained in M2. However, we found that the  $\delta^{18}\text{O}_{\text{sulphate}}$  values differed by  $\sim 1.2\%$  when the results of M1 and M3 were compared. Other possible causes for this difference must be considered and are discussed below.

#### 4.2. Contributions from membrane filter paper

All the three methods result in the formation of BaSO<sub>4</sub> that is filtered using mixed cellulose ester or polycarbonate filters. After drying, the BaSO<sub>4</sub> precipitate is scraped off the filter paper. Accidental removal of some filter particles may add variable proportions of O to the sample with a  $\delta^{18}\text{O}_{\text{filter}}$  value ranging between 5.6 and 14.5 ‰, the O isotope ratios of the used filter papers. This effect would likely be most pronounced for samples that yield very little BaSO<sub>4</sub> on the filter paper. However, our results suggest that the differences in methods resulted in a relatively constant difference in the  $\delta^{18}\text{O}_{\text{sulphate}}$  values and not a higher  $\delta^{18}\text{O}$  value that would have been attributed to any filter contamination.

#### 4.3. Effect of acidic eluent

During the elution procedure of sulphate from resins by adding HCl, we observed chemically produced heat in spite of careful acid treatment. The  $\delta^{18}\text{O}_{\text{sulphate}}$  value is not influenced by heat treatment in acidified solutions, which are needed for the precipitation of BaSO<sub>4</sub> in analytical procedures [31]. Also, once sulphate is produced in an aqueous phase, its  $\delta^{18}\text{O}$  value is conserved. Under ambient pH and temperature conditions, the rate of O isotope exchange between sulphate and water is very low [32–35], even in acidic rain with a pH that is approximately 4 [33]. However, at elevated temperatures, there might be the possibility of enhanced O isotope exchange between water and sulphate driving the  $\delta^{18}\text{O}$  values of sulphate higher. Since elution of sulphate from the resins with 3 molar HCl created acidic conditions, we did not heat this solution during the elution procedure and kept the temperature increases to a minimum by gradually adding the acid. Hence, the differences in the  $\delta^{18}\text{O}_{\text{sulphate}}$  values among the three methods in our study are likely not a result of O isotope exchange between sulphate and water.

#### 4.4. Nitrate contamination

Michalski *et al.* [19] showed that in solutions where nitrate/sulphate molar ratios are above 2, the addition of BaCl<sub>2</sub> will co-precipitate some nitrate (a maximum of approximately 7 %) with the BaSO<sub>4</sub>. Thus, if the nitrate has a substantially different O isotope ratio when compared with sulphate (which is the case for atmospheric nitrate), an erroneously high  $\delta^{18}\text{O}_{\text{sulphate}}$  value may be measured due to contributions from co-precipitated nitrate. However, in the solutions used in our experiment from ALW and the non-manipulated watersheds at the HBEF, the nitrate/sulphate molar ratios were always less than 0.2 [20,25,36,37] and, hence, the potential for nitrate contamination is very low. Therefore, the effect of nitrate co-precipitation is unlikely to explain the observed difference in  $\delta^{18}\text{O}_{\text{sulphate}}$  values between M1 and M2/M3 in our samples.

### 5. Conclusion

There were no significant differences between the obtained  $\delta^{34}\text{S}_{\text{sulphate}}$  values using the three methods of sample preparation. We have shown that the three methods used for preparing water samples

for stable isotope analyses of sulphate result in similar  $\delta^{34}\text{S}_{\text{sulphate}}$  values within an uncertainty of  $\pm 0.3\text{‰}$ . Using the same preparation methods, the obtained  $\delta^{18}\text{O}_{\text{sulphate}}$  values differed occasionally by more than  $1.0\text{‰}$ . To help understand and eliminate the analytical discrepancies associated with the determinations of  $\delta^{18}\text{O}$  values of sulphate, we suggest the following:

- (1) examining internal laboratory standards with known  $\delta^{18}\text{O}_{\text{sulphate}}$  values and  $\delta^{34}\text{S}_{\text{sulphate}}$  values pretreated by the same procedure as samples (e.g. Std A and Std B used in this study);
- (2) introducing and verifying a sample preparation procedure providing detailed information for reducing all possible errors in terms of type of acid for elution, materials of the filter paper used, and related chemical background information; and
- (3) providing information on the solution matrix that may affect O and S isotope ratios of sulphate including concentrations of DOC, nitrate, and sulphate.

In the meantime, we discourage from interpreting differences in the  $\delta^{18}\text{O}$  values of sulphate of less than  $1.5\text{‰}$  for aquatic samples obtained in watershed studies. Further testing of a purification procedure for  $\text{BaSO}_4$  that may improve the accuracy of  $\delta^{18}\text{O}$  measurements of sulphate [38] is also recommended.

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