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Methodology for Analyzing Dimethyl Sulfide and Dimethyl Sulfoniopropionate in Seawater Using Deuterated Internal Standards

Geoff C. Smith,* Terry Clark, Linda Knutsen, and Elaine Barrett

Environmental Sciences Department, CBD, Defence Evaluation & Research Agency, Haslar, Gosport, PO12 2AG, U.K.

A method is presented that provides good accuracy and precision for the determination of dimethyl sulfide (DMS) and its precursor dimethyl sulfoniopropionate (DMSP) in seawater for trials-based equipment. By using deuterated internal standards of DMS-d_6 and DMSP-d_6, the precision for replicate determinations was shown to be as high as 1.6% for DMS and 5.8% for DMSP when the internal standard concentration differed by up to 1 order of magnitude from the components being determined. The method for DMS using "commercial off the shelf" equipment gave a detection limit of 0.03 nM and was linear to >100 nM. The DMSP method required slight modification to the equipment and gave a detection limit of 0.3 nM and was linear to >1000 nM. The most appropriate sample preparation methodology for storing the samples for up to 56 h during intensive sampling periods included filtration, acidification, and refrigeration.

Dimethyl sulfide (DMS) is a major volatile organosulfur compound produced in seawater by certain groups of phytoplankton. It has been suggested that the flux of DMS to the marine atmosphere could affect the cloud albedo and, therefore, the global environment, via oxidation reactions to form cloud-condensation nuclei.[1] Due to the significant spatial and temporal variations in the concentration of DMS in seawater,[2−5] and hence the flux to the atmosphere, there is a need to fully categorize DMS and its precursors in seawater in order to provide more accurate determinations of the global sulfur cycle, especially for its impact on the global climate.[1]

DMS is derived primarily from the enzymatic hydrolysis of dimethyl sulfoniopropionate ($(CH_3)_2S^+CH_2CH_2COO^-$; DMSP), an osmoregulatory compound produced by a wide variety of marine phytoplankton.[2,6] Intracellular DMSP hydrolysis has been shown in phytoplankton,[7] in macroalgae,[8] and also in bacteria following uptake of DMSP from the seawater.[9] Reported seawater concentrations of dissolved DMS (<0.1−90 nM) and DMSP (1−1000 nM) vary with increasing depth, spatially from coastal areas to the open ocean, and also temporally from winter to summer.[2−5]

The method presented here for analyzing DMS in seawater while at sea uses a "commercial off the shelf" purge and trap GC/MS system which, for the DMSP (particulate and dissolved) determinations, required only minor hardware modifications. Most of the current methods analyze the full range of DMS concentrations found in seawater by various purge and cryogenic trap techniques.[10−13] However, the apparatus is often composed of hardware components of various sources of manufacture which are constructed by the research scientists into an optimal configuration for their analyses of DMS in seawater. The benefits of using commercial equipment, however, include its relatively short startup time and the ready comparison of results between laboratories with similar equipment during intercalibration exercises. Intercalibration exercises between "noncommercial equipment" setups have already been demonstrated.[14] The mass spectrometer also adds the potential to identify uncharacterized components in the seawater samples. Conversely, the use of "commercial off the shelf" equipment to this specification does incur greater costs of a factor of 3−4 over more standard setups with a simple cryotrap and GC/FPD.

The methodology reported here uses deuterated internal standards to compensate for component losses during sample preparation, storage, extraction, preconcentration, and analysis. Otherwise the losses of volatile components from the sample container by evaporation or reaction at active sites can cause sources of error. Sample collection and transfer to the sample containers before the internal standards are added are also expected to provide additional sources of error due to factors such as pressure changes and the presence of zooplankton; however, such errors are outside the specification of any sampling methodology and would only be overcome when real-time, in-water

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detection methods have been developed. The deuterated standard DMS-d_6 has been shown to have a Henry's law constant similar to that of the nondeuterated DMS compound.[15] Deuterated standards therefore offer the capability of improving the precision and accuracy of the DMS and DMSP determinations. Deuterated standards also offer the possibility of shorter purge times because component losses from inefficient purging are also reflected in the standard losses.

This analytical method also addresses the problems associated with the storage of seawater samples before DMS and DMSP analysis because intensive sampling periods can often lead to large sample backlogs. This is an important factor to address because the subsequent hydrolysis of DMSP in the seawater by bacteria[16] and possibly extracellular enzymes[11] can cause the apparent DMS concentration to change with increased storage time.

EXPERIMENTAL SECTION

Preparation of Deuterated Standards. Deuterated DMS-d_6 was purchased from Aldrich Chemical Co. (Gillingham, Dorset, U.K.) at 99.5% ^{2}H atom purity. DMSP was prepared from the reaction between DMS and acrylic acid in toluene at room temperature for 4 days before precipitation with hydrogen chloride gas.[17] The precipitate was collected onto a GF/F filter paper, washed with anhydrous toluene, and dried. The DMSP product (yield ~50%) was confirmed by NMR and elemental analysis (C, H, S, Cl: calculated percentage by weight 34.98, 7.06, 18.68, 20.65; found 35.21, 6.60, 17.56, 19.59, respectively). Deuterated DMSP-d_6 standards were prepared by a similar technique using the appropriate starting materials. The contamination of the deuterated compound with nondeuterated compound was determined to be <0.5% for DMS-d_6. Therefore consideration must be made for the contribution to the DMS signal when high concentrations of internal standards are used.

Preparation of Internal Standards. All glassware used for standards and sampling was silanized with dimethylchlorosilane (Sigma Chemicals Co., Gillingham, Dorset, U.K.) in dry toluene[18] and all lids were Teflon lined. Primary standards were prepared with ethylene glycol,[19] which had previously been purged with zero grade nitrogen. Primary standards were prepared gravimetrically[15] while on land and volumetrically while at sea for DMS and DMSP in the parts per thousand range (mg of DMS/g of methanol and mg of DMSP/g of water). A secondary standard containing both DMS and DMSP (μg/g of water) was used to prepare the working standards for calibration purposes (ng of DMS and DMSP/g) and also served as the internal standard stock solution. The Teflon mini-inert valves (Alltech Assoc., Carnforth, Lancashire, U.K.) on the standard vials were used to facilitate the transfer by syringe and kept the interaction of the DMS standard with the atmosphere to a minimum. The variability in the internal standards delivered originated from the error in reading the weight or volume of the standard additions. Negligible losses of DMS were observed from the secondary standard over a 24-h period. Both primary and secondary standards could be made daily.

Sampling. An internal standard containing deuterated DMS-d_6 and DMSP-d_6 was transferred from a 14-mL amber vial fitted with a Teflon mini-inert valve using a syringe (100 μL) and mixed with each sample immediately after collection. For samples that were supplemented with phytoplankton, the internal standard was added to every 30-mL subsample. For seawater samples collected during sea trials, the internal standard was added to each 1-L sample before a 30-mL subsample was removed for DMS/DMSP analysis. The 30-mL samples were filtered with very gentle pressure (3−5 kPa) through a GF/F (glass fiber) filter with a nominal initial retention size of 0.7 μm (Whatman, Merck, Leicestershire, U.K.) held within a sealed 47-mm filtration unit (Nalgene, Merck, Leicestershire, U.K.). The design of the filtration units minimized losses of volatile components by minimizing the air/water interaction. Finer grade filters (0.2 μm, Whatman) were also used with some of the development work. The filtrate was immediately collected in 30-mL amber glass vials containing 75 μl of 2 M sulfuric acid, and the vials with screw top PTFE/silicone septa were stored in cold water (~3 °C) in the refrigerator to minimize loss of the volatile components during long storage periods.

DMS Analyses. The water samples were analyzed for DMS by injecting 25 mL of the acidified filtrate through the Luer inlet port of the glass sparging unit of a Hewlett-Packard/Tekmar purge and trap 3000 concentrator (P&T). The P&T was fitted with a Supelco Vocarb 3000 Carbopack B/Carboxen 1000&1001 trap (type K) interfaced to a Hewlett-Packard (Stockport, Cheshire, U.K.) gas chromatograph (model 5890, series II) with a 9-m, HP1, 0.25-mm-i.d. capillary column and mass selective detector (MSD model 5972) operating in single ion mode for typically 25 ions. The P&T-GC/MS was permanently seated in the base unit of a larger aluminum transit box (Light Alloy Ltd., Ipswich, U.K.). This system has been successfully used on trials at sea and at a coastal station. The P&T conditions were 8-min purge at 50 °C, trap temperature 35 °C during purge (using liquid carbon dioxide turbocool), 250 °C during desorb, and 270 °C during the 1-min bake. The transfer line from the P&T was interfaced into the carrier gas line of the GC injector. The septum purge was blocked and the sample split in the injector liner (7:1). The 25-mL glass sparger was washed between runs with 50 mL of HPLC grade water prepurged with zero grade nitrogen, and blanks were performed periodically. Good resolution was achieved using CP grade helium carrier gas (1 mL/min^{-1}) and with the GC oven at 60 °C isothermal with transfer line, injector, and detector at 280, 250, and 280 °C, respectively. The column was conditioned usually at the end of each working day by slowly ramping the temperature (2 °C/min) from 60 to 250 °C (hold for 1 h). Typically, 60 samples were analyzed for DMS per day during sea trials.

DMSP Analyses. Dissolved DMSP ($DMSP_{(d)}$) was determined by cold hydroxide decomposition of the "DMS-free" prepurged, filtered, acidified sample. A 5 M KOH solution was degassed with zero grade nitrogen, and 10 mL was sealed in each 30-mL glass reaction vial. The "DMS free" filtrate (10 mL) was added through the Teflon/silicone septum using a syringe and needle after first removing 10 mL of headspace. The vials were inverted and stored in a refrigerator. DMSP associated with the particulate matter ($DMSP_{(p)}$) was collected on the GF/F filter papers and analyzed by placing the filter paper in the 30-mL vial

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Table 1. Effect of Filtration on the DMS Response of a Prepared Standard Solution of DMS and DMS-d_6

	peak area (DMS[62,63,64])		peak area (DMS-d_6[68,69,70])		DMS detd (nM)[a]	
sample no.	nonfiltered	filtered	nonfiltered	filtered	nonfiltered	filtered
1	28 193	34 141	29 338	33 680	0.87	0.92
2	32 671	32 472	33 664	32 637	0.88	0.90
3	35 009	31 006	34 755	30 316	0.92	0.93
4	33 417	19 858	35 358	20 585	0.86	0.88
5	35 127	33 575	34 676	33 237	0.92	0.92
mean	32 883	30 210	33 558	30 091	0.89	0.91
std dev	2823	5910	2436	5470	0.03	0.02
CV[b]	8.59	19.56	7.26	18.18	3.02	2.27

[a] Concentration data determined using DMS-d_6 as an internal standard. [b] CV, coefficient of variation (%) ((std dev/mean) × 100).

and adding 20 mL of prepurged 2.5 M KOH. The standards were shown to be completely hydrolyzed within 30 min, and the hydrolysis solutions were shown to be stable for periods in excess of 18 h. Therefore, to maximize DMSP hydrolysis from within the particulate matter, the seawater samples were typically allowed to hydrolyze overnight before the DMS product was analyzed. The DMS product from either the particulate or dissolved DMSP fraction was collected in a glass syringe by purging zero grade nitrogen gas (100 mL at 100 mL/min) through the vial using a long needle. The vial containing the hydrolyzing solution was placed in a water bath at 60 °C for this process. This transfer of DMS was not quantitative, being only ~60% efficient, but this was compensated by the use of deuterated internal standards. The contents of the 100-mL syringe were injected directly onto the trap in the purge and trap unit via an additional "T" piece placed between the glass sparger and the trap using low-volume fittings. This modification allowed the transfer of the gaseous syringe contents via a Luer-lock fitting and a two-way tap directly onto the carboxen trap of the P&T. The DMSP method required only a 1-min purge time which was sufficient time to introduce the sample onto the carboxen trap. It was necessary to adopt a lower GC temperature starting at 25 °C to resolve the DMS product from other components produced from the seawater hydrolyzing solution. The retention time for the deuterated and nondeuterated DMS products was ~0.7 min.

Recent measurements have shown that DMS can also be quantitatively determined by quickly sparging zero grade nitrogen through the sample (1 min) and introducing the sample using a syringe directly onto the carboxen trap (1 min) before analysis (2 min). The detection limit for DMS for this faster method was shown to be 0.3 nM, and replicate analyses of a 1.8 nM standard gave a mean of 1.6 nM and coefficient of variation (CV) of 4.8%. The deuterated standards therefore allow potentially faster analysis times because the DMS does not need to be quantitatively sparged from the solution.

Peak Integration and Calculation. The small presence of naturally occurring isotopes of carbon ^{12}C (^{13}C), sulfur ^{32}S (^{33}S, ^{34}S, ^{36}S), and hydrogen ^{1}H (^{2}D) can form a significant proportion of the total dimethyl sulfide in the environment. Ridgeway et al.[15] determined the isotopic proportions of the DMS molecules in ambient seawater at 92.86% 62 m/z ion, 2.87% 63 m/z, 4.16% 64 m/z, 0.09% 65 m/z, and 0.02% 66 m/z. The molecular ions monitored on the MSD were 62, 63, and 64 m/z for DMS and 68, 69, and 70 m/z for DMS-d_6. These ions were shown in this study to represent at least 99.9% of the total component in the DMS and DMSP standards and samples analyzed. Peak areas were determined using the ion count over the integrated mass range (i.e., 62−64 for DMS).

The concentration of DMS and DMSP in the seawater samples was determined using the following equation:

$$C = \mathrm{PA}^{62-64}\mathrm{IS}/\mathrm{PA}^{68-70}$$

where C is the concentration (nM) of DMS or DMSP being determined, PA^{62-64} is the peak area of the DMS being determined using the integrated ion count of 62, 63, and 64 m/z ions, IS is the concentration, once added to the sample, of the deuterated internal standard (nM), and PA^{68-70} is the peak area of the deuterated internal standard using the integrated ion count of 68, 69, and 70 m/z ions.

Phytoplankton Culturing. Cultures of *Emiliania huxleyi* (920/2) and *Pleurochrysis carterae* (961/8), which are known DMS producers, were obtained from CCMS Dunstaffnage Marine Laboratory, Oban, Scotland, U.K. The freshly collected seawater samples used during the method development period were spiked with cultures of phytoplankton to bring the algal numbers up to those found in the surface ocean under bloom conditions during spring and summer months (~10^4−10^5 cells/mL). Phytoplankton supplements were used when in their log phase of growth. These mixtures were allowed to stand for at least 2 h before use.

RESULTS AND DISCUSSION

Effect of Filtration on DMS Recovery. It was considered necessary to filter the seawater samples before injection onto the P&T as the presence of biota in the P&T glassware may require greater periods for washing and running blanks, and the biota present in the sample container may affect the DMS/DMSP concentration during periods of increased storage time. Before the effect of filtration on seawater samples was addressed, the effect was determined on standards of DMS and DMS-d_6 made up in water. Table 1 shows the DMS and DMS-d_6 data for samples either filtered before analysis on the P&T or analyzed directly on the P&T without prior filtration. It is evident from Table 1 that greater variation of the peak area data was observed for the filtered standards (i.e., 19.6 and 18.2%) compared to the nonfiltered standards (i.e., 8.6 and 7.3%) for both DMS and DMS-d_6. This was primarily the result of a low peak area result reported for the

fourth sample. A *t*-test for paired means, however, showed that there was not a significant difference between the filtered and nonfiltered treatments at the 95% confidence level for the DMS peak area data. By using the DMS-d_6 as an internal standard for the DMS determination it is possible to account for losses that are common to both the DMS and deuterated internal standard. The benefit of using an internal standard can be fully appreciated when the unexpected low DMS peak area of the fourth sample in Table 1 is shown to also be reflected in the low peak area of the DMS-d_6 internal standard. The reason for the low yield of DMS and DMS-d_6 in this sample was unknown, and unusual, but serves to emphasize the advantage of using deuterated internal standards. The DMS concentration was calculated for the filtered (0.89 nM) and nonfiltered (0.91 nM) samples to be comparable to the actual concentration present (i.e., 0.92 nM). Statistical analysis of the concentration data using a *t*-test for paired means also showed that the effect of filtering did not cause losses of DMS at the 95% confidence level.

Effect of Filtration on DMS Determined in Seawater Spiked with Phytoplankton. The effect of filtration was also tested on a fresh seawater sample spiked with a culture of *E. huxleyi* to mimic phytoplankton bloom conditions in seawater. To keep the storage time for each of the filtered and nonfiltered treatments to a minimum, it was necessary to analyze aliquots of the seawater/phytoplankton mixture consecutively over time. DMS concentrations for the nonfiltered samples were 76, 77, and 81 nM for analysis times of 1, 75, and 145 min, respectively. For the filtered samples, DMS concentrations of 53, 49, and 37 nM were found for analysis times of 15, 90, and 165 min, respectively. Therefore, the effect of sparging the culture through a 25-mL fritted sparger without prior filtration for this experiment caused ~53% more DMS to be determined than for the filtered samples. The high increase in DMS in the nonfiltered samples may be a function of the artificially high phytoplankton concentrations used, which were typical of bloom conditions. Other studies have shown that it is either unnecessary to filter seawater prior to purging[3,20] or that filtration is required.[11,12] The filtration of water samples has been reported to affect DMS concentrations under certain conditions[3] whereas purging nonfiltered samples can lead to an apparent 2-fold increase in DMS.[11] Whether these differences are purely down to subtleties in the sampling and analysis technique of each research group or due to differences in the seawater samples analyzed cannot be ascertained at this stage but may suggest a need for further intercalibration exercises.[14] The information presented in this paper suggests that filtration prior to analysis is necessary for the equipment, methods, and samples used.

Effect of Grade of Filtration on DMS Concentration over Storage Time. It was also necessary to determine how the concentration of DMS varied over increasing storage time. Of the research groups who filter the seawater samples, some filter immediately prior to analysis but after storage;[11] however, if the storage time is expected to be greater than 1 h then it may be necessary to filter before storing the samples.[20] For a 14-min turnaround of samples for DMS analysis in the P&T sparger it was expected that periods of intensive sampling could lead to large sample backlogs and long storage periods. Therefore it was considered necessary to consistently filter the samples upon collection and prior to storage in the refrigerator. To test the effect of the grade of filtration, a fresh seawater sample was spiked with a phytoplankton culture of *P. carterae* and filtered through 0.7- and 0.2-μm filters. The 0.7-μm filter was expected to remove most of the phytoplankton and the 0.2-μm filter most of the bacteria. The 0.7-μm and 0.2-μm filtrates were stored in the refrigerator and analyzed over time. Figure 1 shows how the DMS concentration of the stored filtrates of the seawater/phytoplankton mixture varied over time.

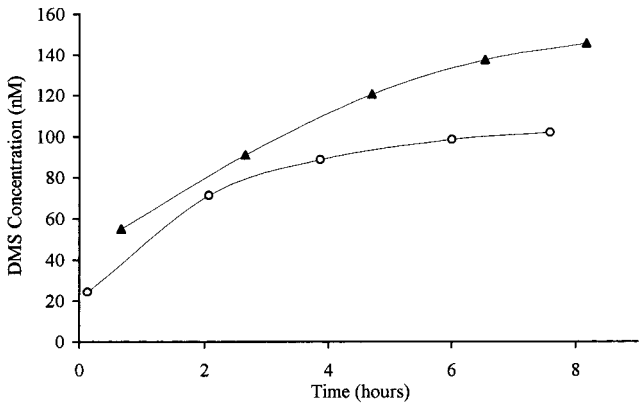

Figure 1. Effect of storage time on the DMS concentration of seawater samples prefiltered through a 0.7-μm (▲) and a 0.2-μm filter (○).

It is evident from Figure 1 that the DMS concentration in both filtrates increased with increasing storage time. The 0.7-μm filtrate showed a greater increase in DMS than the 0.2-μm filtrate, which may be due to a greater abundance of bacteria hydrolyzing DMSP in the seawater.[9] The increase in DMS observed for the 0.2-μm filtrate has also been shown to occur in other studies[3,16] and may have arisen from some cells passing through the filter where acid stress may have caused DMS release. Alternatively, there is a possibility that soluble DMSP lyase enzymes are present in the seawater sample, either naturally or possibly as a result of cell rupture caused by filtration.[16]

Effect of Acidifying Filtrates on DMS Concentration over Increasing Storage Time. Because of the production of DMS in the filtered samples over time, it was necessary to find a means of inhibiting the enzyme DMSP lyase in order to prevent increases in the DMS concentration over increased storage time. DMSP lyase activity has been shown to be negligible below pH 5.[21] The effect of acid additions on the DMS concentration over increased storage time was therefore examined on 0.7-μm filtrates of a mixture of fresh seawater spiked with a phytoplankton culture of *P. carterae* to a total phytoplankton concentration of 10^5 cells/mL^{-1}. It was necessary to filter the samples in order to prevent dissolving the calcium carbonate structure of the phytoplankton that are primarily responsible for DMSP production. Figure 2 shows the effect of acidifying the filtrates on the concentration of DMS over time. Each solution was typically less than pH 3. This experiment was also repeated on the following day and showed comparable results which are also presented in Figure 2. The DMS concentration remained constant over at least the first 56 h

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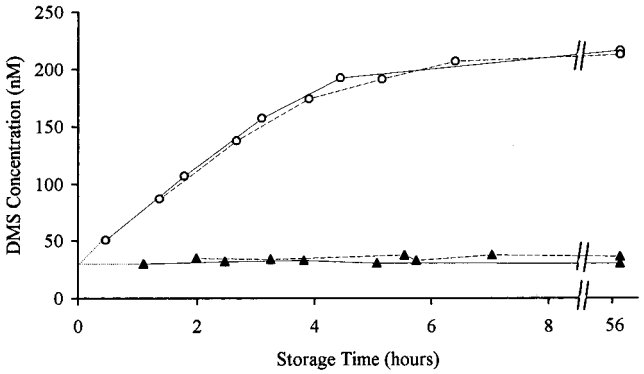

Figure 2. Effect of acidification on DMS concentrations over time for GF/F filtered seawater spiked with phytoplankton. Replicate acidified (▲) and nonacidified (▲) samples are shown.

Table 2. Calibration of DMS Standards Using the Internal Standard DMS-d_6

DMS concn	DMS-d_6 (int std) concn (nM)	peak area: DMS[62,63,64] ions	peak area: DMS-d_6[68,69,70] ions	DMS detd (nM)[a]
160.9	15.5	11382365	1099143	160.5
115.9	15.5	7786344	1052805	114.6
64.4	15.5	4286781	1040997	63.8
32.2	15.5	2018841	960088	32.6
16.1	15.5	1118584	1026623	16.9
7.1	1.3	553325	101567	7.1
2.9	1.3	202773	97915	2.7
1.4	1.3	102887	102223	1.3
0.56	0.11	34275	6571	0.57
0.28	0.11	15916	6215	0.28
0.17	0.11	9942	6392	0.17
0.11	0.11	5969	5588	0.12
0.06	0.11	3160	6397	0.05
0.03	0.11	1748	5827	0.03

[a] DMS determined using the DMS-d_6 internal standard. (e.g. 7.1 nM; (553325 × 1.3)/101567 = 7.1 nM).

of storage for acidified samples, whereas for the nonacidified samples, the DMS concentration increased over time, as was also observed in Figure 1. Regression analysis of the acidified samples gave significantly smaller DMS production rates (−0.10 and 0.35 nM/h) compared to the nonacidified samples (36.3 and 25.6 nM/h). The CVs of the DMS concentration in the acidified samples over the full 56-h period were 4.1 and 6.1% for the two experiments. Extrapolation of the graphs showed the DMS concentration in the samples at the time of sampling ($t = 0$) to be ∼30 nM (see Figure 2), which after 56 h had increased to ∼210 nM for the nonacidified samples and had changed little for the acidified samples. These results therefore suggest that the samples should be filtered, acidified, and refrigerated before analysis, especially for high abundances of DMS-producing phytoplankton.

Calibration and Precision of the DMS Determination. Table 2 represents DMS calibration data for the concentration range 0.03−161 nM. Internal standard concentrations of 15.5 nM DMS-d_6 for the 16.1−161 nM DMS range, 1.3 nM DMS-d_6 for the 1.4−7.1 nM DMS range, and 0.1 nM DMS-d_6 for the 0.03−0.56 nM range were used. The response was shown to be linear over this range of over 3 orders of magnitude ($r = 0.99$), and the detection limit was ∼0.03 nM. Independent reviews of the MSD have shown it to have a detection limit of 2−11 pg of S/s and a linear dynamic range of $>10^5$.[22] The accuracy of the results using this method has been shown to be greatest when the concentration of the internal standard approaches that of the component being determined. Due to the <0.5% contamination of DMS within the DMS-d_6 internal standards, care must be taken to avoid using higher than necessary internal standard concentrations. For example, a 6 nM internal standard concentration would raise the concentration of the DMS being determined by up to 0.03 nM.

Five replicate analyses of the following DMS standards, 0.11, 2.86, and 16.1 nM, gave determined means of 0.11 (±0.002, CV = 1.9%), 2.64 (±0.05, CV = 1.8%), and 16.58 (±0.26, CV = 1.6%) nM, respectively. Precision was typically reduced to ∼2.5% as the internal standard differed from the components being determined by up to 3 orders of magnitude.

DMSP Hydrolysis. A wide range of hydroxide concentrations have been quoted in the literature to promote the hydrolysis of DMSP to DMS, such as 0.25 M NaOH,[23] 1 M NaOH,[24] 1 M KOH,[9] and 1.6−2.5 M NaOH.[12] To test whether this affected the amount of DMS hydrolyzed from DMSP and DMSP-d_6, a range of hydroxide solutions were prepared at 0.25, 0.5, 1.0, and 2.5 M KOH final concentration and analyzed over increasing reaction time. The hydrolysis of the DMSP internal standard and the natural DMSP present in the seawater was 90−100% complete within 30 min for the 1.0 and 2.5 M KOH solutions. The 0.25 M KOH solution required at least 4 h before complete hydrolysis was achieved. The DMS hydrolysis product of the 2.5 M KOH solution was typically stable for at least 18 h; therefore, most reactions could commence overnight with determinations made the following day. It should be noted that DMS can be produced from a variety of organosulfur precursor compounds,[17] but in the oceans it appears to be derived mainly from DMSP.[16] However, the relative proportions of the various DMS precursors in seawater that form DMS upon hydrolysis have yet to be presented in the literature.

DMSP Calibration and Precision. A calibration range of 0.7−2280 nM DMSP using DMSP-d_6 internal standards of 7, 35, and 350 nM for this range was linear with a correlation coefficient of $r = 0.99$. Table 3 shows the DMSP calibration which compares gravimetric amounts to DMSP determined using the DMSP-d_6 internal standard.

Five replicate analyses of DMSP standards of 14.2, 54.5, and 436 nM gave determined means of 13.2 (±0.9, CV = 6.9%), 53.8 (±3.1, CV = 5.8%), and 433 (±30, CV = 6.9%) nM. The DMSP detection limit for the current method using a 10-mL filtered seawater sample is ∼0.3 nM.

During sea trials, the greatest variation in DMS and DMSP concentration is typically found with increasing depth where concentrations can range from as high as 90 nM DMS and 1000 nM DMSP near the sea surface to less than the detection limit of this method at greater depth (i.e., <0.03 nM DMS and <0.3 nM DMSP).[2−5] Spatial variations encountered in the upper ocean (i.e., 0−20 m) during single studies typically show less variation in DMS and DMSP of between 1 and 2 orders of magnitude.[2−5,21]

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Table 3. DMSP Calibration Comparing Gravimetric to Calculated Determinations Using the DMSP-d_6 Internal Standard

DMSP concn (nM)	DMSP-d_6 (int std) concn (nM)	peak area		DMSP detd (nM)[a]
		DMSP[62,63,64]	DMSP-d_6[68,69,70]	
2280	350	2949865	437306	2361
1266	350	1787802	490984	1274
633	350	501504	274905	639
545	350	772092	503826	536
436	350	572928	445659	450
327	35	364494	36816	347
218	35	200727	30812	228
109	35	121042	39272	108
54.5	35	46971	30033	54.7
28.4	7	34107	7186	33.2
14.2	7	11456	5561	14.4
7.1	7	8426	8027	7.4
3.6	7	2651	5303	3.5
1.8	7	2264	8445	1.9
0.7	7	713	6840	0.7

[a] DMSP determined using the DMSP-d_6 internal standard. (e.g. 14.2 nM; $(11456 \times 7)/5561 = 14.4$ nM).

The single most appropriate internal standard concentration to choose at sea to account for these depth-related variations in DMS and DMSP would be 6 nM DMS and 60 nM DMSP. Thereby good precision and accuracy of the results would be expected for samples collected from the surface ocean, which is necessary for accurate flux calculations to the atmosphere. Also, at greater depths, where the DMS and DMSP concentration approaches the detection limit of our method, the contribution of contaminant DMS (i.e., <0.5%) present within the deuterated internal standard would also remain negligible though the accuracy and precision may be slightly decreased at these depths.

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