

Laboratory inter-comparison of dissolved dimethyl sulphide (DMS) measurements using purge-and-trap and solid-phase microextraction techniques during a mesocosm experiment

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Abstract

We compare dissolved dimethyl sulphide (DMS) measurements made by our independent laboratories during a mesocosm study of marine phytoplankton under different CO₂ regimes in a Norwegian fjord. Sample preparation and analyses were conducted using headspace solid-phase microextraction (SPME) with gas chromatography-mass spectrometry (Max-Planck Institute for Chemistry, MPIC), and purge-and-trap extraction (P&T) with gas chromatography and flame photometric detection (University of East Anglia, UEA). The two analytical systems were calibrated independently. During the evolution of the bloom (22 days) DMS concentrations ranged from 1–35 nM and 90 pairs of data were available for comparison. We found a small systematic difference between the two methods, with UEA measuring on average 8% more DMS than MPIC. Overall, there was good correlation between the datasets ($r^2=0.997$, $P=0.01$), with higher correlation for concentrations greater than 5 nM ($r^2=0.998$, $P=0.01$) and increased scatter at lower concentrations ($r^2=0.833$, $P=0.01$). We discuss potential reasons for the differences between the measurements and address the treatment of natural samples for DMS analysis. We recommend SPME be considered for wider use and encourage full analytical comparisons in the low concentration range.

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1. Introduction

DMS is a volatile sulphur compound produced in marine ecosystems. DMS has been proposed as a counterfoil to global warming through the formation of

cloud condensation nuclei from its oxidation products and their effect on the radiative forcing of the atmosphere (Charlson et al., 1987). DMS concentrations in seawater are usually below the detection limit of most detectors and it is therefore necessary to extract and pre-concentrate a sample, prior to analysis. A frequently used technique for extracting dissolved gas from aqueous samples both from the natural ocean and from laboratory culture studies is the purge-and-trap method

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(P&T). Here, DMS is extracted from the liquid phase by purging the sample with an inert gas such as nitrogen or helium and then passing the gas stream through a cryo-trap where the DMS is captured while the nitrogen passes through. The condensed DMS is then thermally desorbed and injected into a gas chromatograph (GC) for detection and quantification using, for example, flame photometry (Turner et al., 1990). Manual P&T is laborious and time-consuming and is usually the time-limiting step in a series of sample analyses. Water samples have to be analysed for DMS as soon as possible after sample collection, because biological activity or exposure to light can alter the DMS concentration. Similarly, the addition of preservatives to water samples can cause disruption of algal cells and the release of DMS. Thus, water samples cannot be stored on the longer term, which can be disadvantageous for open ocean sampling, where the space for equipment is sparse and time for analysis of samples may be limited. An additional consideration of the P&T method is that both filtering and purging of the sample may be disruptive to some organisms (Kiene and Slezak, 2006; Niki et al., 2000), leading to *in situ* DMS production. Since the latter is considered the greater problem, water samples are generally filtered prior to analysis, although here again the action of filtering could compromise the measurement to some extent as biomass accumulates under pressure on the filter. Recently, a method has been developed that could potentially avoid many of the problems associated with the P&T technique. The solid-phase microextraction (SPME) technique uses a retractable SPME fibre contained inside a septum-piercing needle (Arthur and Pawliszyn, 1990; Belardi and Pawliszyn, 1989). The fibre is coated with either a liquid polymer or solid sorbent, depending on the application. The needle is injected into the headspace above or into a liquid sample and is left there for a period of equilibration, during which time the target compounds are adsorbed or absorbed and concentrated on the surface of the fibre. The fibre can then be injected directly into the GC injection port where the compounds are released by thermal desorption. Thus, SPME is a sampling technique causing only minimal disturbance of the sample. SPME has been applied successfully to the extraction of volatile sulphur compounds, including DMS (Haberhauer-Troyer et al., 1999; Mestres et al., 1998; Niki et al., 2004; Yassaa et al., 2006) as well as for other environmentally significant organic species such as monoterpenes (e.g. Yassaa and Williams, 2007). Furthermore, Sakamoto et al. (2006) show that SPME fibre samples can be stored and preserved for up to 20 days after sampling without significant changes in DMS concentrations. Thus, SPME would appear to be suitable for open

ocean sampling of DMS, with some potential for systematic sampling and the generation of extensive data sets.

In this work we present the results of an inter-laboratory comparison between the University of East Anglia (UEA) and the Max-Planck Institute of Chemistry (MPIC), involving independent measurements performed on identical, natural sub-samples of seawater containing DMS. We compare the results obtained by using the 'classical' purge-and-trap technique (UEA) with results obtained from SPME facilitated measurements (MPIC). We show where differences in the measurements occur and comment on the future use of both techniques.

2. Methods

2.1. Nature of the dataset

DMS measurements were taken during a mesocosm CO₂ enrichment study conducted in a Norwegian fjord, close to Bergen (PeECE III), in May and June 2005. The experimental set-up consisted of 9 polyethylene enclosures (*ca.* 20 m³, 9.5 m depth) filled with unfiltered fjord water and adjusted to 3 different pCO₂ concentrations (375 ppmV, 750 ppmV, 1150 ppmV), with triplicate bags for each treatment. A phytoplankton bloom was triggered in each enclosure *via* the addition of nutrients (0.7 μM PO₄, 15 μM NO₃) and the developing blooms were studied over a period of 24 days. Details of the set-up and procedures can be found in Engel et al. (2005). The analytical equipment for DMS measurements (UEA and MPIC) was set-up in a laboratory at the mesocosm facility and all DMS measurements were performed on site. Details of the data reported here, and further results obtained for the three different pCO₂ treatments can be found in Vogt et al. (2007), or in Sinha et al. (2007) and Wingenter et al. (2007).

2.2. Sampling for DMS measurements

Sampling of all mesocosm bags was carried out daily using 5 L polyethylene aspirators. Prior to sampling, all aspirators were thoroughly rinsed first with natural fjord water and then with water from the respective mesocosms. The mouths of the aspirators were then covered with a 200 μm mesh filter in order to exclude mesozooplankton grazers and the aspirator taps were left open to release air during sampling. Each inverted aspirator was slowly immersed, down to a depth of approximately 0.3 m, allowing 200 μm screened water to enter the container. When approximately 3 L of water had been collected, the aspirator tap was closed and the aspirator was then righted and capped off. The bulk samples were then transported to a cold room, where the aspirators were kept at *in situ* water temperature (9–11.5 °C) and in dim light. Sub-samples for all trace gas measurements were taken from these aspirators.

2.3. DMS measurements by Purge and Trap (UEA, M. Vogt)

DMS was measured using a 'triple' P&T system for cryogenic enrichment of DMS (Turner et al., 1990) in combination with gas chromatography. Immediately prior to sampling, the 5 L aspirators were gently rotated to ensure sample homogeneity and then sub-samples of seawater were taken using Teflon tubing and gas-tight syringes. Sampling was completed within 2 h after collection from the enclosures. The samples were directly injected into the purge tube, through a glass-fibre filter (25 mm, Whatman GF/F) contained in an in-line holder: slow, gentle pressure was applied to the syringe in order to minimise disruption to the phytoplankton cells trapped on the filter. The analytical volumes (5–18 mL) were adjusted during the course of the experiment to accommodate changes in concentration. Samples were purged for 15 min (N_2 , 60 mL min^{-1}) during which time the DMS collected in a Teflon tubing loop, maintained at $-150 \text{ }^\circ\text{C}$ (temperature controlled headspace above liquid nitrogen). Thereafter, the condensate was thermally desorbed by immersing the cryo-loop in boiling water and the sample flushed into the split/splitless injector of a Shimadzu GC-2010 gas chromatograph with $30 \text{ m} \times 0.53 \text{ mm}$ CP-Sil 5 CB capillary column and a flame photometric detector. The GC oven was run isothermally at $80 \text{ }^\circ\text{C}$ and DMS eluted at 1 min. Since the time-limiting step in the sample analysis is the period of purging, by using three separate P&T systems, each connected to a sample stream selection valve, sample throughput was significantly increased. Full calibrations of each P&T system were carried out every 3–4 days, with daily spot checks before sample analyses. Working standards were made using a sterile stock solution of dimethylsulphoniopropionate (DMSP, CAS, The Netherlands) diluted with MilliQ water. Volumes analysed (3 or 13 mL) reflected the volume of the samples and the concentration range was 0.3 to 24.3 nM. 82% of the samples were within this concentration range and additional calibrations after the field study confirmed the linear behaviour of detector response beyond 35 nM DMS. For analysis, 1 mL of NaOH solution (500 mM) and 1–12 mL MilliQ water were added to the purge tube and the mixture was bubbled for a few seconds, before the N_2 flow was turned off and the DMSP standard solution was injected. Thereafter, the procedure was the same as for sample analysis. The alkaline cleavage of DMSP to DMS and acrylate is completed within a few minutes (White, 1982). The detection limit in 18 mL of sample was less than 0.3 nM DMS and the analytical uncertainty was 6%.

2.4. DMS measurement by solid-phase microextraction (MPIC, N. Yassaa)

DMS was measured using headspace solid-phase microextraction and gas chromatography-mass spectrometry (HS-SPME-GC-MS), see Yassaa et al. (2006) for details. Briefly, a manually operated SPME holder and a $65 \text{ }\mu\text{m}$ polydimethylsiloxane-divinylbenzene (PDMS-DVB) coated SPME fibre (Supelco, Germany) were employed. Sample preparation was conducted in the cold room in order to maintain the water samples at the same temperature as in the mesocosms. Glass

vials (20 mL) containing 10 mL of unfiltered water sample were equilibrated for 1 h, to allow partition of DMS between the water and headspace phases. After this time, DMS was extracted from the vials by centrally piercing the septum with the protected needle and exposing the fibre to the headspace, 2 mm above the water for 1 h. Initial tests showed that equilibrium and exposure times of 1 h each gave optimal results at sample temperatures of $9\text{--}11.5 \text{ }^\circ\text{C}$.

Calibration checks for each SPME fibre were performed during the course of mesocosm experiments at temperatures of $9\text{--}11.5 \text{ }^\circ\text{C}$ with one point standard solution (5 nM of DMS in water) and corrected *a posteriori*, after further method development, with a multiple point calibration, adopting the procedure described in Yassaa et al. (2006). Briefly, a DMS solution of $50 \text{ }\mu\text{M}$ was prepared in chromatography-grade deionised water (Merck, Darmstadt, Germany) and dilutions were made immediately afterwards. Aliquots of 10 mL of each DMS solution (ranging from 0.005 nM to $50 \text{ }\mu\text{M}$) were dispensed and sealed in 20 mL vials fitted with PTFE/Silicone septa and the standards were immediately subjected to SPME extraction. In order to further reduce measurement uncertainty, we recommend the use of filtered, autoclaved degassed water for future studies.

Immediately after extraction from a water sample or calibration standard the fibre was introduced into the split/splitless injector of the gas chromatograph. A glass inlet liner with a narrow internal diameter (0.75 mm i.d. , Supelco) was used in order to improve the GC resolution and the peak shape. Desorption was achieved in splitless mode at $200 \text{ }^\circ\text{C}$ for 2 min.

Analyses were conducted using a gas chromatograph (Agilent Technologies, GC 6890A) coupled to a Mass Selective Detector (MSD 5973 *inert*) from the same company. The MSD, with an electron impact source running in SIM mode (monitored m/z was 62 for DMS), was operated with the following conditions: ionization potential 70eV and source temperature $230 \text{ }^\circ\text{C}$. The DMS peak was resolved using a β -cyclodextrin capillary column (CYCLODEX-B, 30 m-long, 0.256 mm ID , $0.25 \text{ }\mu\text{m}$ film thickness) supplied by J & W Scientific (California, USA). Following the introduction of the SPME needle to the GC injector and the analytes entering the column, the column temperature was maintained at $40 \text{ }^\circ\text{C}$ for 5 min, then increased to $200 \text{ }^\circ\text{C}$ at $1.5 \text{ }^\circ\text{C min}^{-1}$ and finally held at this temperature for 5 min. For the mesocosm samples, it was found that no further compounds were eluted during the second half of the temperature programme, so analyses were terminated after 1 h. With a helium (Messer Griesheim 6.0) carrier gas flow rate of 1 mL min^{-1} , the retention time of DMS in the chromatogram was 1.64 min. The precision was 8% and the detection limit was less than 0.5 nM, for the experimental conditions described above. It is important to note that lower detection limits are achievable but this depends *inter alia* on temperature and the favourability of the air–water partition coefficient. It was found that the efficiency of one SPME fibre decreased significantly after about 100 uses, so fibres were discarded after 90 extractions. SPME fibre extraction of DMS was linear for a concentration range of 0.05 nM to $5 \text{ }\mu\text{M}$ (Yassaa et al., 2006).

Since the aim of the SPME analyses was to examine a large number of compounds, the long GC temperature programme was required. Thus, in order to complete the daily analyses, samples were taken from two of the triplicate enclosures for each CO₂ treatment.

3. Results

The mesocosm experiment fortuitously provided the opportunity to conduct a laboratory inter-comparison of the

analysis of natural samples which covered a wide range of DMS concentrations (1–35 nM). The dataset discussed in this work comprises all the replicate analyses (UEA and MPIC) from the daily sampling of six enclosures, for which there are 90 pairs of data. Fig. 1 shows how the DMS concentrations varied with time and visual comparison of the results from the two measurement techniques suggests rather close agreement for individual enclosures (M1 to M8).

The temporal evolution of DMS concentrations showed clear differences between enclosures and between pCO₂

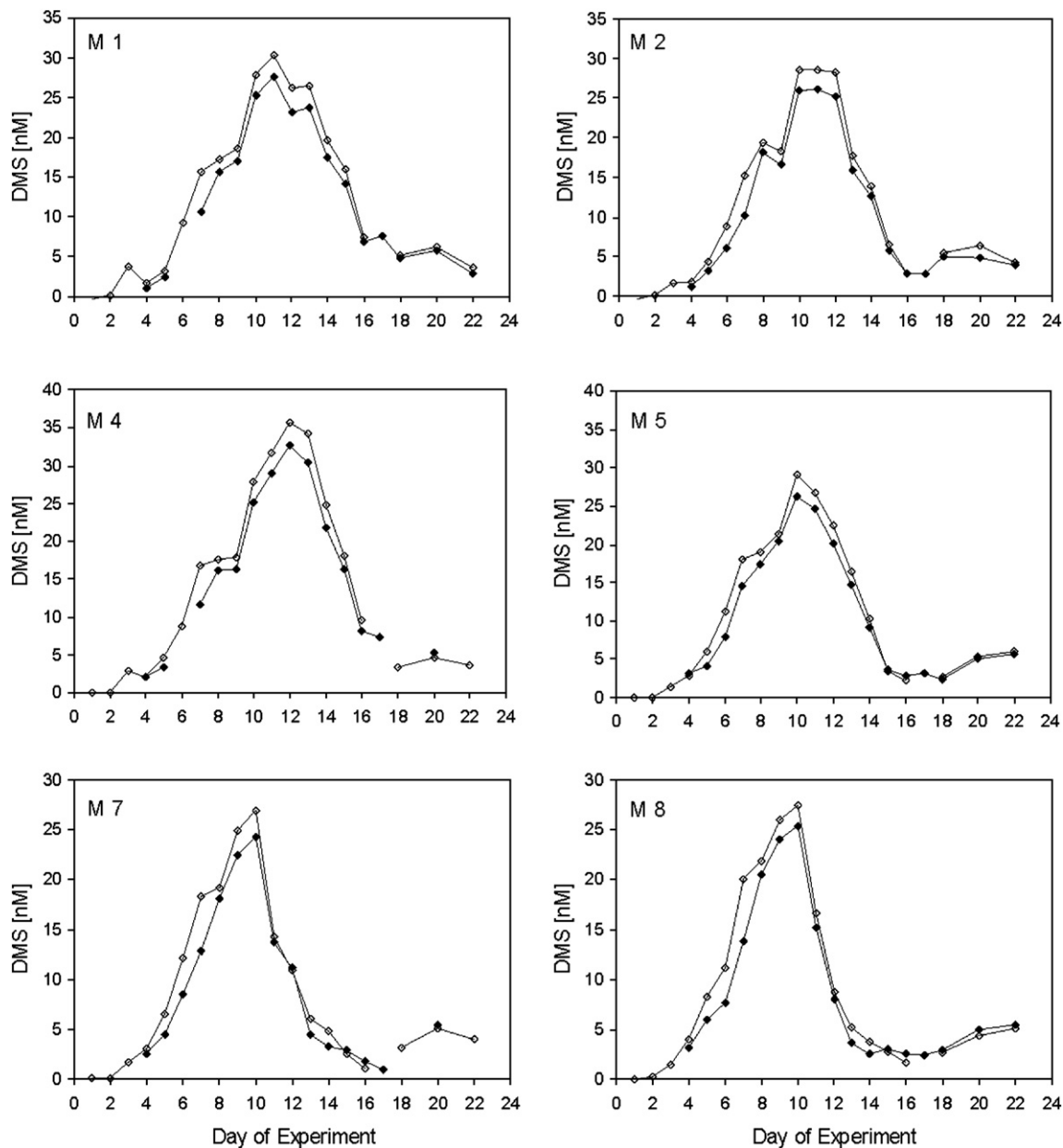


Fig. 1. Temporal variation in DMS concentrations during the mesocosm experiment. Measurements using SPME (filled symbols) and P&T (open symbols) for individual mesocosm bags: M1, M2, present day pCO₂; M4, M5, double pCO₂ and M7, M8, triple pCO₂.

treatments. However, analysis of our findings for the different treatments is not within the scope of the work presented here: for more information see Vogt et al. (2007).

3.1. Statistical analysis

The two data sets show similar means (SPME: 11.86 nM, P&T: 12.93 nM) and variances (SPME: 8.78 nM, P&T: 9.49 nM), but both sets deviate strongly from normal distribution (One sample Kolmogorov–Smirnov Test, SPME: $\sigma=0.007$, P&T: $\sigma=0.013$) and the data are almost in rank order. Therefore, the use of appropriate rank tests (such as the Mann–Whitney U test) for non-normal distributed data will not reveal existing differences between the two data sets. Hence, we use the mean of the differences (SPME–P&T) to detect random error and bias. The differences are reasonably normally distributed (One sample Kolmogorov–Smirnov test, $\sigma=0.743$). Their mean is 1.06 nM with a standard deviation of

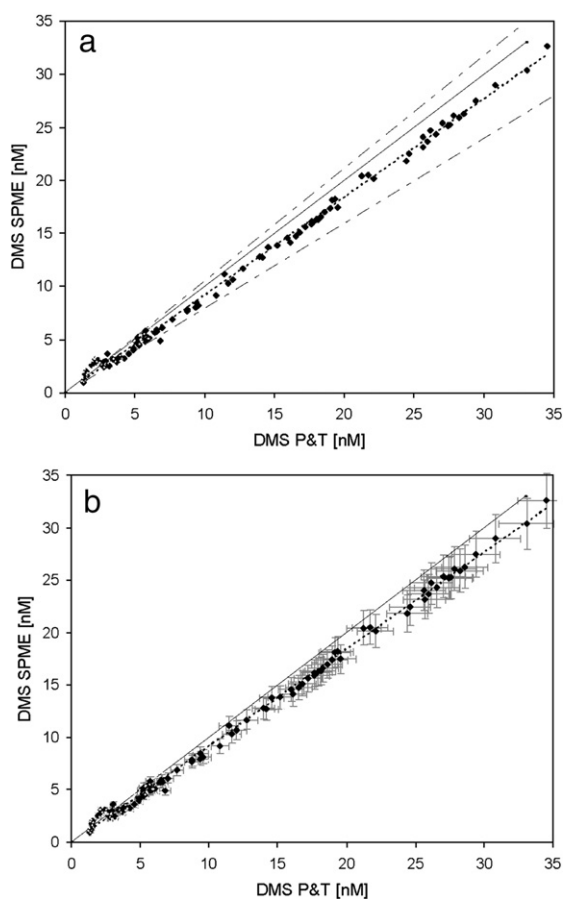


Fig. 2. a) Correlation of DMS measurements from MPIC (SPME) and UEA (P&T). Data pairs are represented by diamonds. The dotted line is the best linear fit curve for all data pairs. The dashed lines show the envelope of uncertainty of the data, based on analytical precision. The solid line represents the ideal 1:1 relationship for the measurements. b) Data pairs (diamonds) including uncertainty bars and 1:1 relationship (solid line). Bars indicate errors based on analytical uncertainty only.

0.86 nM. A one-sample t -test shows that the difference of the data is significantly different from 0 ($P < 0.001$). Hence, we conclude that there is a small systematic difference between the two methods.

In the following, X – Y plots and regression lines are investigated for the different concentration ranges. Fig. 2a shows the correlation of the DMS concentrations measured using P&T and SPME. Within the concentration range covered by our measurements, the datasets are strongly correlated ($r^2=0.997$, $P=0.01$), with higher correlation for concentrations greater than 5 nM ($r^2=0.998$, $P=0.01$) and increased scatter at lower concentrations ($r^2=0.833$, $P=0.01$). Linear regression of the data (SPME / P&T) gives a curve with slope of 0.924 (orthogonal regression: 0.925), which is less than the ideal value of 1. Based on the spread of the data, regression analysis of the best-fit curve gives an approximate value range for the uncertainty in slope and intercept. The results of the ANOVA technique show, firstly, that the intercept of the fitted curve is -0.076 ± 0.083 (orthogonal regression: intercept = -0.09), which indicates only a small offset between the sets of measurements. This suggests that there was no consistent background contamination or DMS loss, for either of the analytical techniques. Secondly, since the uncertainty in the slope of the curve is 0.924 ± 0.006 and its maximum value is still less than 1, there is a small, but significant systematic difference between the datasets. When the analytical uncertainties of 8% (SPME) and 6% (P&T) are taken into account, the significance of the difference from the 1:1 line is decreased (Fig. 2b). This is shown by the slopes of the best-fit curves through the upper (mean slope = 1.06) and lower envelopes (mean slope = 0.88) of the data including the analytical errors (Fig. 2a), which can exceed 1. Fig. 2b shows the data including analytical error bars, but without best-fit curves.

3.2. Concentration dependence of difference

Since the datasets spanned a relatively large concentration range, we now examine their co-relation as a function of concentration. Fig. 3 shows the difference (P&T–SPME), normalised to the mean concentration of DMS measured and plotted against the mean concentration. We use mean DMS concentration on the x axis to avoid any inference that one dataset is better than the other. As is to be expected, there is increasing scatter in the relative differences with decreasing concentrations. For 88% of the paired data, UEA measured higher concentrations than MPIC and, further, this was always the case for mean concentrations above 5.8 nM. Furthermore, 78% of the differences lie within 30% of the mean DMS concentrations.

4. Discussion

4.1. Data comparison

The main objective of the mesocosm experiment was to determine the biogeochemical response to different

pCO₂ regimes and it was fortuitous that co-participation in the study enabled comparison of our DMS measurements. Due to this fact, it was not possible to perform a full analytical comparison of the two methods using the same equipment and identical standards for the calibration of the methods. The inter-laboratory comparison of our DMS measurements in natural seawater samples has allowed us to address two issues. Firstly, we believe that the results show that our laboratories are able to make very comparable measurements for a wide range of concentrations. This finding helps provide some justification for the quality of the data generated by our separate laboratories. Since there is no certified standard for DMS, we cannot assess the accuracy of our measurements. However, the techniques we use are very different (e.g. sample preparation, type of calibration standard and analytical instrumentation), which may indirectly confer some degree of analytical accuracy.

The second issue is an assessment of the application of SPME for DMS measurements in seawater samples containing the mixed populations of a natural community, at different levels of biomass. In the current literature there are a few reports on the use of SPME for extraction of DMS from natural seawater samples (e.g. (Niki et al., 2004; Sakamoto et al., 2006; Xiaoying et al., 1993)). However, none of these studies included comparison of SPME with other methods of sample preparation. Statistical analysis of the two datasets showed only small systematic differences between the two methods and UEA tended to measure about 8% more DMS than MPIC. This could have arisen from inaccuracies in the preparation of the different calibration standards but this is speculative since we did not analyse each others standards.

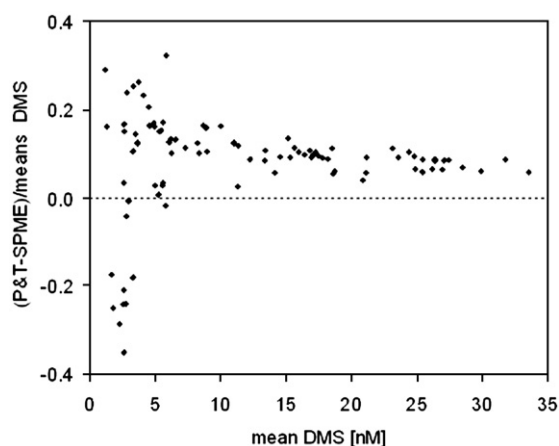


Fig. 3. Differences between the SPME and P&T DMS datasets normalised to mean concentrations versus mean concentration. Differences lie mostly in the range of $\pm 30\%$.

4.2. Assessment of potential artefacts in SPME and P&T analyses

The slope between the two measurements lies within the [0.90, 1.10] interval which is the best class of systematic error (Chemical Co-ordinating Centre of EMEP, 1996). However, there is mechanistic potential for the sample preparation techniques to produce different concentrations. The following discussion addresses some of the issues. Higher measured concentrations can occur if there is *in situ* production of DMS during handling, filtering and purging of water samples. Each process imposes physical stress on the sample which may lead to increased activity of DMSP-lyases and generation of DMS. Further, lyases released into a filtered sample can remain active for periods much longer than that of analysis. Such artefacts are more likely to occur with P&T since more stress is applied than with SPME.

DMS can also be lost during sample preparation, most obviously by exposure to the atmosphere but also by surface adsorption. This phenomenon is more likely with P&T, since DMS is exposed to a far greater surface area than in the small vials used with SPME. Such loss is accounted for when calibration standards are treated in exactly the same way as samples, i.e. purging of aqueous standards rather than direct injection of gas standards. Under-estimation of DMS can also be associated with the efficiency of SPME extraction: the fibre coating has a small volume and the limited number of adsorption sites can become saturated. Additionally, for a sample containing a cocktail of gases, there is competitive adsorption between DMS and other volatiles with high affinity for the sorbent. This occurs by direct competition for site occupation and by DMS molecules being ousted by other compounds with stronger affinities. To minimise the latter, fibre exposure times are kept as short as possible. Yassaa et al. (2006) developed the SPME method applied here in a study of laboratory cultures and found that 1 min exposure time was optimal. However, owing to the low temperature of the mesocosm samples, the exposure time was increased to compensate for the slow rate of transfer of volatiles from the water phase to the headspace. We do not have any evidence that competitive adsorption could have caused MPIC to measure slightly lower concentrations than UEA.

4.3. Assessment of SPME and P&T techniques for in-field use

With regards to the selection of either SPME or P&T for the analysis of field samples, there are additional

considerations. Sample preparation with P&T is normally the time-limiting step in the analytical process which limits the number of samples that can be collected simultaneously (i.e. storage for more than a few hours is not recommended). SPME, however, can enable rapid analytical throughput, and fibres can be stored for up to 20 days without significant loss of DMS (Sakamoto et al., 2006).

This is advantageous when it is not possible to run analyses immediately. However, SPME fibres have to be individually calibrated and monitored for performance, which is time-consuming. Additionally, fibres have a commercially recommended lifetime of only 100 uses and, although Niki et al. (2004) suggest capability of a few hundred extraction–desorption cycles, we found 90 extractions to be a safer limit. For P&T the most likely restriction on the number of samples analysed is the lifetime of the 6-port switching valve which is used to inject the sample onto the GC column.

From the results of our data comparison alone, we cannot judge the suitability of either SPME or P&T for environments with predominantly low concentrations of DMS. In the global ocean, 95% of all measured concentrations are less than 6 nM (Kettle and Andreae, 2000), which is the level below which our datasets show increasing disagreement. Although our detection limits were very similar (MPIC, 0.5 nM; UEA, 0.3 nM), UEA methodology had been optimised for the higher concentrations expected during the mesocosm bloom. That is, different sample volumes (and purge times) help compensate for the limited dynamic range of the Flame Photometric Detector response. Thus, with P&T, there is scope to improve the detection limit and precision for lower concentrations. With SPME, some improvement in detection limit can be achieved by decreasing the volume ratio of headspace to water sample (Yassaa et al., 2006). However, with very small headspaces there is risk of the fibre being wetted by the water sample and this is a particular consideration when using the technique on a ship.

In summary, both techniques have advantages and disadvantages and selection of either depends to a large extent on the nature of the study environment, the number of samples, access for immediate analysis, equipment restrictions and the expected range of DMS concentrations.

5. Conclusion

SPME is a relatively new technique for the determination of DMS concentrations in natural water and is attractive since there is minimal disturbance of the

samples. SPME has been used in laboratory culture studies (Yassaa et al., 2006) and has been applied to analyse natural samples (Niki et al., 2004; Sakamoto et al., 2006). It has been recommended for in-field use by these authors. We showed that independent measurements of DMS with both SPME and P&T yield similar results and there appeared to be only a small systematic difference between the results. However, full assessment of sample preparation by P&T and SPME requires a custom study where common equipment and calibration standards are used in the analyses. In particular, such a study should focus on the DMS concentration range found in open ocean environments, where we found the largest deviations between the two methods. We concur with other investigators who recommend SPME for field use (Niki et al., 2004; Sakamoto et al., 2006) and we are hopeful that its application will help enable more systematic and extensive measurement of DMS which is required for better information on global distributions, seasonal cycling and the underlying biological mechanisms governing the production of this climate active gas. As such, SPME could be beneficial to both the experimental and modelling DMS communities.

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