Dimethyl sulfide (DMS) derived from marine biological activity affects radiative forcing of the climate. The general analytical technique for DMS in seawater (purge and trap method, P&T) is complex onboard ship. Thus it is difficult to obtain sufficient data for a comprehensive understanding of the spatiotemporal variability of DMS in the sea surface layer. On the other hand, a new analytical method for DMS using SPME (solid-phase microextraction) has recently been developed as an alternative method to P&T. This method is simpler than P&T because no special or complex apparatus is needed. If it is possible to preserve DMS for an extended period in excess of the duration of the cruise, the SPME method is a promising method for measuring DMS in seawater. We assessed an analytical method which can allow us to preserve DMS on the long-term scale using SPME. In liquid nitrogen (−196 °C), as preserved environment, for a period of 20 days after sampling, we found the preservation rate of DMS to be 94.7 ± 4.4% (n = 6) in this study. Furthermore, estimating the distribution coefficient with respect to the effect of salinity on SPME, we found that DMS changed by 0.1 nM/% sal, suggesting that salinity has only a minor influence on oceanic DMS measurements in the open ocean because the minimal change of the open ocean salinity is within 2%. Applying the SPME method to open ocean samples, we found that there were no significant differences in DMS between the unpreserved and preserved samples (r = 0.99, n = 26, SE = 0.01, p < 0.0001), showing the SPME method has potential for use for open ocean surveys.

Dimethyl sulfide (DMS) is a volatile sulfur compound produced by marine biota, which has been hypothesized to partly counteract greenhouse warming through its impact on atmospheric chemistry and radiative balance.1 Much attention has been focused on describing the concentration of DMS in the sea surface layer.2–6 Furthermore, to clarify the impact of each process related to DMS on climate change, it is necessary to obtain a comprehensive understanding of the variability of DMS production, thus requiring extensive data sets. However, the general analytical technique for DMS,7 the purge and trap method (P&T) requires the measurement of DMS to be carried out quickly because there are some problems (the effects of biological activity and light intensity) in preserving DMS in seawater samples. Thus, DMS must be measured as quickly as possible within 24 h after sampling.7 To measure DMS in pelagic ocean samples, it is generally necessary to use a large research vessel to facilitate the use of large complex measuring devices (gas chromatograph-flame photometric detector, GC-FPD) and H2 gas. In actual observations, it is difficult to clarify the seasonality of DMS production in all sea areas by large research vessel surveys only. Most observations rely on short period observations using small and midsized research vessels. In general, these vessels enable extensive observations of the pelagic ocean but do not have sufficient space for analytical instruments. For example, we used a vessel with 466 ton and 50 m in the tonnage and the length, respectively. In these vessels, it is difficult to load the required large equipment for the P&T method. Under such a situation, the available data were insufficient to show the detailed distribution of surface DMS in the global ocean. If it is possible to preserve and bring back DMS samples to the laboratory, we can overcome the above problems.

Niki et al.8 developed SPME (solid-phase microextraction8–12) as a DMS analytical method to replace the P&T method for seawater. SPME is a method in which the seawater sample is made to be in the gas–liquid equilibrium condition in a thermal incubator (25 °C). The SPME fiber is then exposed in the headspace of the vial for 5 min to extract the DMS onto the SPME fiber.
fiber under static conditions at 25 °C. The extracted DMS is then immediately desorbed thermally in the injection port (250 °C), separated, and detected by GC/MS directly. This is simple without requiring an complicated apparatus as in the P&T and GC-FPD methods. After extraction on the SPME fiber, if it is possible to preserve the DMS for the duration of the cruise; therefore, it will not be necessary to use a large research vessel, and it is possible to obtain many DMS data.

Using the SPME method described by Niki et al., we assessed the potential for long-term preservation for DMS. Because it is necessary to consider the distribution coefficient of DMS in the gas–liquid phases related to the salinity effect in SPME, we also discuss this effect.

EXPERIMENTAL SECTION

Standard Solution of DMS. According to Niki et al., we made the solution of dimethylsulfoniopropionate (DMSP) chloride dissolved in 0.5 M NaCl as a stock DMS solution. An aliquot of 18 mL was dispensed into a 30 mL glass headspace vial (Maruemu no. 6, 34 mL total volume), and 2 mL of 5 M NaOH was added into the glass vial to hydrolyze DMSP to DMS.

Apparatus, Analytical Condition, and Analytical Procedure. We show a schematic diagram of the instruments used in this study and the analytical procedure for DMS by using SPME (Figure 1 and refs 8–12). In the shaking bath, standard solutions of DMS of 0.2, 1.8, 5.0, 8.0, and 10 nM diluted from the stock DMS solution were achieved under the gas–liquid equilibrium condition during 30 min at a water temperature of 25 °C. To extract DMS from seawater, we exposed the SPME fiber in the headspace of the glass vial for 5 min under static conditions at 25 °C. For the SPME fiber, we used a fiber coated with a combination of carboxen and poly(dimethylsiloxane) (PDMS) (Supelco), and its thickness was 85 μm. Removing only the SPME fiber units from the holder for only the preserved samples, we stored the fiber units in a centrifuge tube (Eiken Kizai Co. Ltd.) and placed the centrifuge tube in liquid nitrogen for 0–20 days in the long-term preservation experiment. We used a preserved period of 20 days here because in our opinion the sailing period for small to midsized research vessels is a few days to a few weeks. Thus, we set a ceiling of 20 days as the preserved period. The DMS extracted on the SPME fiber was desorbed thermally in the injection port (heated at 250 °C) of the GC (GC-17A, Shimadzu) and carried in the capillary column (TC-624, 60 m × 0.25 mm i.d./1.40 μm film, GL Sciences Inc.) with He gas at a flow rate of 1.0 mL min⁻¹. We detected DMS with a mass spectrometer (QP-5000, Shimadzu) using the SIM mode (selected ion monitoring) (monitored m/z (mass/charge) values were 62 and 47).

Comparison between the DMS Content of Preserved and Unpreserved of Samples Using SPME. We developed a supporting experiment to assess the possibility of preserving DMS in samples taken during oceanographic surveys. On the R/V Hokko-Maru of the Hokkaido National Fisheries Research Institute (Oyashio wide investigation period, 2005.01.11–19, 2006.01.19–02.03; Figure 2), we collected natural seawater samples from 9 layers between 0 and 150 m at each of three observational points using 10 L Niskin bottles mounted on a CTD system. We drew seawater samples into 100 mL glass syringes without air bubbles and placed them in a cooler box until the beginning of the following procedures. All samples were measured within 24 h. First, an aliquot of 20 mL was filtered through a glass fiber filter (Whatman GF/F) into the 30 mL vial; we immediately sealed it using a septum and an aluminum cap. We shook the vial bottle in a thermal incubator for 10 min. In the headspace of the vial bottle, we exposed the SPME fibers to extract DMS for 5 min under static conditions at 25 °C. After the above treatment, we immediately measured DMS as unpreserved DMS samples onboard the research vessel. The preserved samples were immersed in liquid nitrogen (−196 °C), and we measured them at the shore laboratory as long-term preserved DMS samples.

Salinity Effect Experiment. We used solvent with the different salinity values (0, 14, 21, and 35%) for dilution. Diluting 1 μM DMSP solution and 2 mL of 5 M NaOH with the above solvent, we prepared DMS solutions of 2, 5, 10, and 15 nM in 30 mL glass headspace vials (34.7 mL total volume). Then, we hydrolyzed DMSP to DMS. According to the analytical procedure described above, we estimated the effect of salinity on DMS with the SPME method.

Figure 1. Procedure for measuring DMS by the SPME method.

Figure 2. Location of the DMS sampling stations in the western North Pacific.
RESULTS AND DISCUSSION

Long-Term Preservation of DMS using SPME. We made the calibration curve for the preserved samples before and after the preservation experiment. We show the results as time series data for 20 days in Figure 3. These results are represented as the average value of three different fibers (±SE). This efficiency was 97.55 ± 0.05% for the longest preservation period of 20 days, and the entire total efficiency was 94.7 ± 4.4% during the period of 20 days. However, there was a lower preservation efficiency with a larger error for the other days (maximum SE of ±9.3%). This may be caused by some factors: low coefficient of determination of the calibration curve \( r^2 = 0.94 \) compared to the normal calibration curve \( r^2 = 0.999 \) and the low sample number \( n = 3 \). However, considering replicate precision of our method of 6% \( n = 9 \) and the above errors, we conclude that the preservation of DMS in liquid nitrogen is feasible as 94.7 ± 4.4% for a term of 20 days by the SPME method.

Salinity Effect on DMS Content by Using SPME. The results of salinity effect\(^{13}\) for the prepared experimental samples, which were performed the DMS concentrations of 2, 5, 10, and 15 nM and salinities of 0, 14, 21, and 35% are shown in Figure 4a. The salinity effect was large with an increase of DMS content. For the DMS concentration of 15 nM, we found the linear trend of the salinity effect to be 0.1 nM/% salinity (Table 1). Because the change of salinity is within 2%, the salinity effect on DMS in the open ocean was less than 0.2 nM, which is small compared to our replicated precision of 0.9 nM for a DMS concentration of 15 nM. We thus conclude that it is not necessary to correct for the salinity effect when measuring DMS using SPME in the open ocean. However, in the coastal area where salinity changes may be more significant, it may be necessary to make a correction for the salinity effect using this SPME method. On the basis of the results of the salinity effect studies, shown in Figure 4a, we can express the following equations for correction of the salinity effect.

\[
\text{DMS}_{\text{cor}} = \text{DMS}_{\text{obsd}} + \Delta\text{DMS}\Delta\text{sal}
\]

where DMS and \( \Delta\text{DMS} \) represent the concentration of DMS and the salinity effect on DMS \( (\Delta\text{DMS} \text{ nM/% salinity}) \). The subscripts cor and obsd are the corrected and observed DMS values. \( \Delta\text{sal} \) represents \( S_{\text{cal}} - S_{\text{act}} \), in which \( S_{\text{cal}} \) and \( S_{\text{act}} \) represent the salinity when we calibrate each fiber and the salinity of the actual seawater, respectively (Figure 4b).

![Figure 3.](image)

Figure 3. Preservation efficiency of DMS by the SPME method from 0 day to 20 days (see Figure 2). The solid and broken lines represent the efficiency of 100% with an analytical precision of 6%. Each error is the standard error.

![Figure 4a](image)

Figure 4a. Salinity effect on DMS using the SPME method. (a) Measuring the same solution of DMS content, we showed the GC/MS peak area for each salinity: ▲, ●, △, and ○ represent the solvent with salinities of 0, 14, 21, and 35%, respectively. (b) Regression curve in the corrected equation: \( \Delta\text{DMS} = 5.1 \times 10^{-4}\text{[DMS]} + 1.7 \times 10^{-2} \quad (r^2 = 0.95, n = 12) \) (nM/% salinity).

<table>
<thead>
<tr>
<th>salinity (%)</th>
<th>DMS (nM)</th>
<th>salinity effect (nM/%)</th>
</tr>
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<tbody>
<tr>
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<td>14.9</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>16.1</td>
<td>0.1</td>
</tr>
<tr>
<td>21</td>
<td>17.1</td>
<td>0.1</td>
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<tr>
<td>35</td>
<td>17.9</td>
<td>0.1</td>
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Table 1. Salinity Effect for 15 nM DMS

In general, the following equation can be used to obtain the actual content of DMS

\[
\text{DMS}_{\text{cor}} = \text{DMS}_{\text{obsd}} + \Delta\text{DMS}\Delta\text{sal}
\]

On the basis of Figure 4b, we estimated the relation between $DMS_{\text{obsd}}$ and $\Delta DMS$.

$$\Delta DMS = 5.1 \times 10^{-3} DMS_{\text{obsd}} + 1.7 \times 10^{-2}$$

$$\left( r^2 = 0.95, n = 12 \right) \quad (2)$$

Using this equation, we can make a correction for the salinity effect on DMS using the SPME method.

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Figure 5. (a) Relative intensity of $m/z$ (47/62) with the change of DMS content. The relationship was not constant around 0.2 nM. (b) An example of correlation between the counted area by GC/MS and DMS concentration for each uncorrected $m/z$ 47 ($\bullet$) and $m/z$ 62 ($\triangle$). (c) An example of the correction for DMS content below 0.2 nM using the regression line of high concentration of above 1.8 nM with the stable ratio ($m/z$ 47, $r^2 = 0.98, n = 4$; $m/z$ 62, $r^2 = 0.97, n = 4$): $\bullet$, $\blacktriangle$, and $\blacktriangleleft$ represent the uncorrected $m/z$ 47 values and the corrected and uncorrected $m/z$ 62 values, respectively. As a regression line, we re-estimated the following equations: $\log(\text{area}) = 2.76 + 1.19 \log(\text{DMS})$ for $m/z$ 47 ($r = 0.99$), $\log(\text{area}) = 2.62 + 1.17 \log(\text{DMS})$ for $m/z$ 62 ($r = 0.99$).

Figure 6. Comparison of the vertical profile of DMS between the preserved and unpreserved samples by the SPME method. We show the vertical profiles of DMS in 2005: (a) station A04, (b) station A07, and (c) station A11 and $\bullet$ and $\Diamond$ represent the results of DMS content for the unpreserved and preserved samples, respectively.
Correction of the Calibration Curve for Lower Concentrations of DMS by GC/MS. In this study, we detected DMS by a mass spectrometer (MS) with an EI (electron ionization) ion source running in SIM mode (monitored m/z values were 47 and 62). In general, we estimated the DMS content as sum of the m/z 47 area and the m/z 62 area while the ratio of 47/62 remained constant. We showed the relative intensity (47/62) as each calibration point in Figure 5a. This relative intensity of above 1.8 nM was constant within ±5%. However, these relative intensities were not constant near 0.2 nM, and the area of m/z 62 was not a straight line, indicating that it is difficult to detect low concentrations of DMS (0.2 nM). This may be the result of the cleavage of the molecule by EI in the MS as the difference in the progress situation of the fragmentation, or it may be caused by a contaminated line in the measuring device, indicating that the ratio of the signal-to-noise is low.

To exclude the unstable relative intensity of 0.2 nM and to apply the other stable relative intensity values from 1.8 to 10 nM, it is necessary to re-estimate the calibration curve. We made a correction for the area of m/z 62 of 0.2 nM in Figure 5b using each m/z recalibration curve. Thus, when we measure a low concentration of DMS, we have to use both SIM and EI modes to check the stability of the GC/MS.

Application of Our Preservation Method of DMS in Natural Seawater. On the basis of the above methods, we measured DMS in the subpolar region of the western North Pacific (Figure 2). We show the vertical profiles of the unpreserved and preserved DMS at stations A04, A07, and A11 in 2005 (Figure 6a, b, and c, respectively). The average DMS concentration for all samples was $0.75 \pm 0.43$ nM ($\pm$ SD, $n = 30$), which is consistent with previously reported values obtained using a general analytical method (P&T, GC-FPD) during the autumn and winter in the western North Pacific subpolar region. We also found no significant differences in the content of DMS between the unpreserved and preserved samples using SPME ($r = 0.99, n = 26, SE = 0.01, p < 0.0001$) (Figure 7), showing that the difference in DMS between the unpreserved and preserved samples was within the SPME analytical error of 6%, and the difference was not significant. In the above analytical method, it took about 20 min for the pretreatment onboard, and we believe that it is possible to further reduce the actual pretreatment time if several samples are simultaneously treated. It took about 10 min back at the laboratory to analyze one preserved DMS sample using GC/MS.

In this study, we calibrated each fiber with a calibration curve. Haberhauer-Troyer et al. previously pointed out that the total analytical time became longer when more fibers were used in an experiment. Using the “extraction rate” as the extraction efficiency of DMS for each fiber (see Supporting Information), we can overcome the criticism about drawing out an analysis time by using multiple SPME fibers. Thus, we conclude that the SPME method is potentially useful, and the long-term preservation during the period of one cruise using the SPME method is applicable to DMS in seawater.

CONCLUSION

We established a feasible method for the long-term preservation of DMS in seawater with a SPME fiber ($\pm 6\%$). Furthermore, we also considered the salinity effect which is the difference of the amount of DMS absorbed on the SPME fiber resulting from the difference in salinity; we showed that the DMS absorbed changed by 0.1 nM/% salinity for high DMS concentrations.

We conclude that this long-term preservation for DMS using SPME method is suitable not only in the western North Pacific subpolar region but also in a wide area of the ocean.

Using our long-term preservation method with SPME, it is possible to obtain DMS data sets from various cruise surveys in the future.

ACKNOWLEDGMENT

We were grateful to the captain and crew of the research vessel R/V Hokko-Maru for their significant assistance in the seawater sampling. We also thank the staff of the Hokkaido National Fisheries Research Institute for their help.

SUPPORTING INFORMATION AVAILABLE

A description of the calibration of the SPME fibers in the DMS measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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Supporting Information (Appendix)

Establishment of long-term preservation for Dimethylsulfide (DMS) by Solid-Phase Micro Extraction (SPME) method

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Abstract

Dimethylsulfide (DMS) derived from marine biological activity affects radiative forcing of the climate. The general analytical technique of DMS in seawater (Purge & Trap analytical method, P&T) is complex onboard ship. Thus it is difficult to obtain sufficient data for a comprehensive understanding of the spatiotemporal variability of DMS in the sea surface layer. On the other hand, a new analytical method of DMS using SPME (Solid-Phase Micro Extraction) has recently been developed as an alternative method to P&T. This method is simpler than P&T as no special or complex apparatus is needed. If it is possible to preserve DMS for an extended period in excess of the duration of the cruise, the SPME method is a promising method for measuring DMS in seawater. We here assessed an analytical method which can allow us to preserve DMS on the long-term scale by using SPME. In liquid-nitrogen (-196 °C) as preserved environment for a period of 20 days after sampling, we found the preservation rate of DMS to be 94.7 ± 4.4 % (n = 6) in this study. Furthermore, estimating the distribution coefficient with respect to the effect of salinity on SPME, we found that DMS changed by 0.1 nM / ‰-Sal, suggesting that there is only a minor influence of salinity on oceanic DMS measurements in the open ocean due to the minimal change of
the open ocean salinity is within 2 ‰. Applying the SPME method to open ocean samples, we found that there were no significant differences in DMS between the unpreserved and preserved samples ($r = 0.99$, $n = 26$, $SE = 0.01$, $p<0.0001$), showing the SPME method has potential for use for open ocean surveys.
Calibration of each SPME fiber in DMS measurement.

In this study, we used the following countermeasure for shortening the number of analytical times required when we used multiple fibers.

In general, the distribution coefficient of DMS in seawater which achieved the gas-liquid equilibrium, is represented as follows by using the Henry’s constant of DMS$^{14}$,

$$H = \frac{p_{\text{DMS}}}{[\text{DMS}]} \quad (1)'$$

where ‘$H$’, ‘$p_{\text{DMS}}$’ and ‘$[\text{DMS}]$’ represent the Henry’s constant, DMS partial pressure (atm) in the gas phase and DMS concentration in the liquid phase (mol/l), respectively.

Expressing ‘$H$’ for seawater to be a function of temperature$^8$,

$$\ln H = \frac{-3547}{T} + 12.64 \quad (2)'$$

where ‘$T$’ represents of seawater sample temperature (K).

In the gas-liquid equilibrium, mass balance equation of DMS can be expressed as follows

for $t = 0$ as the initial condition

$$M_{\text{DMS}(0)} = n_{\text{g}(0)} + n_{\text{l}(0)} = p_{\text{DMS}(0)} \cdot V_g / (R \cdot T) + [\text{DMS}]_{(0)} \cdot V_l \quad \square \quad n_{\text{l}(0)} = [\text{DMS}]_{(0)} \cdot V_l \quad (3)'$$
for \( t = t' \) as the equilibrated condition

\[
M_{\text{DMS}(t')} = M_{\text{DMS}(0)} = n_{\text{g}(t')} + n_{\text{l}(t')} = p\text{DMS}(t') \cdot V_g' / (R \cdot T) + [\text{DMS}](t') \cdot V_l
\]  
(4)

where \( M_{\text{DMS}(0)} \) and \( M_{\text{DMS}(t')} \) represent the total number of moles of DMS in both gas and liquid phases in the time \( t \). \( n_{\text{g}(0)} \), \( n_{\text{l}(0)} \), \( n_{\text{g}(t')} \) and \( n_{\text{l}(t')} \) represent each mole of DMS in the gas and liquid phase in the time \( t \). \( V_g' \), \( V_l \) and \( R \) represent the gas-phase volume, the liquid-phase volume and the gas constant, respectively.

Then we aim to determine the DMS concentration using the “extraction rate” for each fiber to substitute determination of DMS concentration using each calibration curve for each fiber.

We can estimate the ideal expectation of the gas-phase DMS concentration \( n_{\text{g}(t')} / V_g' \) for the prepared sample with a known value using Eqns.(1)’-(4)’,

\[
n_{\text{g}(t')} / V_g = [\text{DMS}](0) \cdot V_1 \cdot H / (V_g \cdot H + V_1 \cdot R \cdot T) \quad (5)
\]

Because all of the above parameters have a known value except \( n \), we easily can estimate

\( n_{\text{g}(t')} / V_g \). Using a calibration curve of DMS standard with each 1µL benzene base standard in the above same condition, we defined the extraction rate as follows

\[
E = [\text{DMS}]_{\text{std}} / (n_{\text{g}(t')} / V_g) \quad (6)
\]
where \('[DMS]_{\text{std}}'\) represents the DMS concentration on the SPME fiber determined by the above calibration curve. According to Eqns. (3)' and (4)', we can show the actual number of moles of DMS \((n_{l(0)})\) in seawater as

\[
n_{l(0)} = n_{g(t')} + n_{l(t')}
\]

\[
= \left(\frac{n_{g(t')}}{V_g}\right) \cdot V_g + \frac{p_{\text{DMS}(t')}}{H} \cdot V_l = \frac{[DMS]_{\text{std}}}{E} \cdot V_g + \frac{p_{\text{DMS}(t')}}{H} \cdot V_l \quad (7)'
\]

We can obtain the DMS concentration in seawater as

\[
[DMS]_{(0)} = \frac{n_{l(0)}}{V_l} \quad (8)'
\]

where \('V_l'\) represents the volume of the seawater sample (20 ml, in this study).

Besides the above, it is also possible to determine the DMS concentration in seawater by the internal standard method. This is one of the GC-MS methods which uses the internal standard substance such as dimethyl-6d disulfide.