Shinya Hashimoto[a], Toshiyuki Tanaka[b], Nobuyoshi Yamashita[b], Tsuneaki Maeda[c]

[a] Department of Ocean Sciences, Tokyo University of Fisheries, 4-5-7 Konan, Minato-ku, Tokyo 108–8477, Japan
[b] National Institute for Resources and Environment, 16-3 Onogawa, Tsukuba, Ibaraki 305–8569, Japan
[c] DKK Corporation, 4-13-14 Kichijoji Kitamachi, Musashino-shi, Tokyo 108-0001, Japan

An automated purge and trap gas chromatography-mass spectrometry system for the sensitive shipboard analysis of volatile organic compounds in seawater

We developed an automated purge and trap unit connected to a gas chromatograph-mass spectrometer for shipboard determination of unstable volatile organic compounds in seawater. The device used a small column for the rapid desorption of adsorbed compounds, thus eliminating the need for post-desorption cryofocusing. The repeatability (relative standard deviation, RSD; $n = 7$) was typically < 5%. The detection limits were 0.1–4.3 pM for chloromethane, bromomethane, dichloromethane, iodomethane, dimethyl sulfide, iodoethane, isoprene, bromochloromethane, chloroform, tetrachloromethane, dibromomethane, bromodichloromethane, iodopropane, chloroiodomethane, dimethyl disulfide, dibromochloromethane, bromoform, and diiodomethane. To investigate the stability of seawater samples, we obtained a concentration-time profile of volatile organic compounds using this method during the incubation of a seawater sample with and without the addition of $HgCl_2$ in the dark at 4 °C. We found shipboard determination to be suitable and essential for the determination of unstable compounds such as dimethyl sulfide in seawater, as the concentration of dimethyl sulfide increased considerably during the incubation of a seawater sample both with and without the addition of $HgCl_2$. This method permitted the assessment of numerous naturally produced volatile organic compounds that are considered to be important for the chemistry of seawater/atmosphere exchange in the ocean.

Key Words: Shipboard analysis; Purge and trap analysis; Mass spectrometry; Seawater; Volatile organic compounds (VOC)

Ms received: September 20, 2000; accepted: November 27, 2000

1 Introduction

Volatile organic compounds (VOCs) produced in the marine environment are thought to play a key role in atmospheric reactions, particularly those involved in the global radiation budget and the destruction of tropospheric and stratospheric ozone [1–3]. Over the oceans, the oxidation of dimethyl sulfide (DMS) is a major source of sulfur dioxide, the principle precursor for atmospheric sulfate aerosols, which scatter incoming radiation [4]. Volatile organic compounds, including halogens and halocarbons that are produced by marine algae and phytoplankton, may cause ozone depletion in the troposphere and stratosphere [5–8]. The assessment of numerous naturally produced VOCs in the atmosphere and in seawater is considered to be important for the estimation of the seawater/atmosphere exchange of these gases in the ocean [9].

Commonly, it is easier to measure VOCs in the atmosphere [10] than it is to measure them in seawater samples. Typically, seawater samples collected for the analysis of VOCs must be preserved and analyzed later in a land-based laboratory. Although halocarbons have reportedly remained stable for many months in environmental water that has been acidified and stored under refrigeration [11], the stability of halocarbons has not been confirmed within a few hours after sampling. The concentration of DMS is less stable, as DMS can be produced by the decomposition of dimethylsulfoniopropionate (DMSP) generated by marine organisms, particularly phytoplankton [12]. The rate of DMS release is greatly increased when the phytoplankton are subjected to grazing by zooplankton [13]. Analysis in a land-based laboratory system may be insufficient to achieve a correct value of unstable VOCs in seawater if samples need to be transported long distances from their point of collection. Recently, many compounds have been measured on board, for example,

Correspondence: Prof. Shinya Hashimoto, Department of Ocean Sciences, Tokyo University of Fisheries, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan.
E-mail: shinyah@tokyo-u-fish.ac.jp
Fax: +81 3 5463 0398

 1615-9306/2001/0202–0097$17.50+.50/0

hydrogen peroxide in seawater [14], trace gases in seawater [15], atmospheric methane [16], and arsenic species in seawater [17]. Moreover, investigators reportedly have used on-site monitoring using sensors to evaluate pCO_2 and O_2 dynamics in surface seawater [18]. The accurate determination of these VOCs in seawater through sample analysis immediately after collection is important.

Hino et al. have described a device that allows headspace gas to be sampled and was used to measure VOCs in water samples [19]. However, the detection limit of this system for both chloromethane and bromomethane was 30 ng/L [20]; this sensitivity is too low to measure the concentrations of these compounds in seawater. For highly sensitive determination of VOCs in seawater, a purge and trap system is a promising instrument. Such a system can also be adapted to automatic measurement with a gas chromatograph (GC) [20–22] through fast and sensitive sample preparation. Volatile compounds are purged through a nitrogen flow followed by collection of the analytes on an appropriate trap [20]. Usually, cryogenic focusing by liquid nitrogen is required for purge and trap methods, and for condensation and post-cryofocusing that are used to enrich the analytes [23]. To eliminate the use of liquid nitrogen on a ship, rapid desorption of adsorbed compounds is needed to eliminate the step of post-desorption cryofocusing. Hino et al. described the measurement of VOCs such as vinyl chloride by rapid desorption of adsorbed compounds with a trapping tube that is smaller than the tube used in the US Environmental Protection Agency (EPA) method [19].

We describe here the results of the first shipboard analysis of VOCs in seawater, with a small column coupled with an automated purge and trap gas chromatograph-mass spectrometer (GC-MS) system. In our previous papers [20, 22], we described the development of an automatic analyzer consisting of a purging chamber, an adsorption and desorption column with a cooling and heating system, and a GC equipped with an electron capture detector (Shimadzu GC 9A-ECD, Shimadzu Co. Ltd., Tokyo, Japan); we used this system to analyze nitrous oxide in seawater. In order to enrich and detect various VOCs extracted from seawater, we examined the use of a small column and mass spectrometer on board a ship. The potential of the MS detector allowed the optimal analysis of a wide range of VOCs detected in seawater samples without cryofocusing on board. The parameters of purge and trap extraction were optimized. This system enabled us to measure highly volatile organic compounds such as chloromethane (bp −24 °C) from the water column of the open ocean within 1 h after sampling without cryogenic focusing using a small sample volume (20 mL).

2 Materials and methods

2.1 Chemicals

All reagents used were of analytical grade. Spiking standards were obtained from Supelco (Tokyo, Japan) and Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Composite working standard solutions at 1, 10, 50, and 100 ng/mL were prepared in methanol. Aqueous standards were prepared by diluting suitable aliquots of composite working standards with ultra pure water (Milli-Q water, Millipore Corporation, Bedford, MA, USA), which was used to decrease the background level of of the composite working standards. Working standard of 1,4-difluorobenzene (4-8944, Supelco, Tokyo, Japan) in methanol was used as an internal standard. A standard gas was also used (10 ppb each of iodomethane, dichloromethane, bromochloromethane, chloroform, dibromomethane, bromodichloromethane, chloroiodomethane, dimethyl disulfide, dibromochloromethane, bromoform, and diiodomethane in nitrogen, and 100 ppb each of chloromethane, bromomethane, DMS, and isoprene in nitrogen produced by Taiyo Toyo Sanso Co. Ltd., Tokyo, Japan); the concentration of this standard gas was checked with another standard gas (Taiyo Toyo Sanso Co. Ltd., Tokyo, Japan). The agreement was within 1.9%.

2.2 Instrument

The self-constructed small adsorption and desorption column with a cooling and heating device, equipped with an automatic analyzer consisting of a purging chamber, HP 5972 GC-MS detector (Hewlett Packard, Tokyo, Japan), and calibration system, is shown in **Figure 1**. To eliminate vibration of the MS on the ship, which shook horribly compared to a land-based laboratory, a vibration-proof stage made with a double rubber layer was used. An adsorption and desorption column (150 mm × 2.17 mm ID × 3.17 mm OD) containing about 0.3 g of Tenax TA (35–60 mesh, Alltech Associatess Inc., Deerfield, IL, USA) with silanized glass wool plugs was used to enrich VOCs with boiling points between −24 °C (chloromethane) and 181 °C (diiodomethane). The thermal desorption unit was connected to an HP 5890 Series II GC. This instrument enabled the direct thermal desorption of a small column without a cryofocusing step. The small column was placed such that the carrier gas flow during desorption was opposite to the nitrogen flow during sampling. The small column was heated from −10 ° to 250 °C at 300 °/min and was maintained at 250 °C for 15 min. To remove moisture from the adsorbed analytes, a magnesium perchlorate trap (10 mm ID × 100 mm length; Wako Pure Chemical Industries, Ltd., Tokyo, Japan) was used in-line. We changed the magnesium perchlorate trap every day.

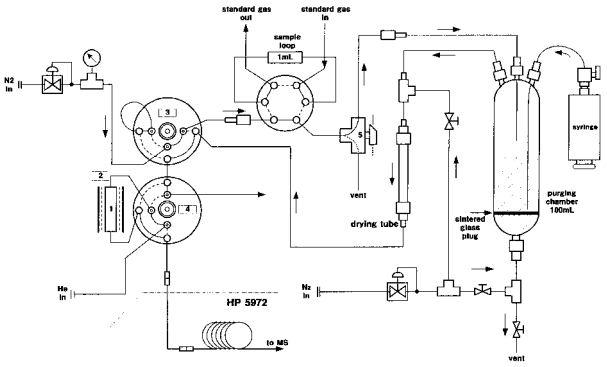

Figure 1. Schematic diagram of automated purge and trap GC-MS system. 1, small column with Tenax TA; 2, heater and cooler; 3 and 4, six-way valves; 5, three-way valve.

2.3 Analytical procedure

Experimental conditions are shown in **Table 1**. At first, the seawater sample (20 mL) and 5 μL of internal standard 1,4-difluorobenzene were injected into the purging chamber with a gas-tight syringe. Then, the VOCs were purged from the seawater with the nitrogen flow, and they were carried to the small trap column, which had been cooled to -10 ± 2 °C. The small column was heated rapidly to 250 °C, and the desorbed VOCs were introduced into the PoraPLOT Q capillary column (cat. no. 7552, Chrompack, Middelburg, Netherlands). The transfer line between the small column and the capillary column was kept at about 200 °C. Flushing the column of Tenax TA with nitrogen gas for 15 min at 250 °C, after desorption had ended, prevented column memory of the tailing compounds. Every day, the small column was conditioned by purging it with nitrogen at 30 mL/min and 250 °C for 2 h before use. Analysis of VOCs was done through selected ion monitoring using GC-MS to obtain high sensitivity. Monitored and qualifier ions and retention times for VOCs are shown in **Table 2**. The concentration of each VOC was obtained with an internal standardization method. The retention time for diiodomethane was 18.95 min (Table 2), and analyses of VOCs could be performed on board within 1 h after sampling.

Table 1. Experimental conditions.

Trapping material	Tenax TA
Purging time	20 min
Purge flow	60 mL/min
Trap temperature	−10 °C
Desorption temperature	250 °C
Capillary column	Chrompack 7552, 10 m (length), 0.32 mm (inner diameter), 10 μm (film thickness) coating PoraPLOT Q
Oven temperature	initial 55 °C for 4 min programmed to 210 °C at 12 °/min
Carrier gas	He
Carrier gas flow rate	1.5 mL/min

Table 2. Monitored and qualifier ions and retention times for the target compounds.

Compound	Monitored ion (*m/z*)	Qualifier ion (*m/z*)	Retention time (min)
Chloromethane	50	52	6.58
Bromomethane	94	96	7.44
Dichloromethane	84	86	10.07
Iodomethane	142	127	10.27
Dimethyl sulfide	62	47	10.42
Iodoethane	156	127	10.42
Isoprene	68	67	11.36
Bromochloromethane	130	128	12.22
Chloroform	83	85	13.83
Tetrachloromethane	119	117	14.00
Dibromomethane	174	172	14.03
Bromodichloromethane	129	127	14.56
Iodopropane	170	43	14.68
Chloroiodomethane	176	178	14.75
Dimethyl disulfide	94	79	15.40
Dibromochloromethane	129	127	16.29
Bromoform	173	171	18.01
Diiodomethane	141	127	18.95

2.4 Calibration of purge and trap

For calibration, each 20 mL of ultra pure water was spiked with the composite working standard at a different concentration in methanol and purged with nitrogen. 1,4-Difluorobenzene was added to the water sample as an internal standard at 1 pg/mL before purging. Calculations were based on the peak area of the individual compound in relation to the peak area of the internal standard. The detection limit was defined as three times the peak height of the blank. Calibration plots for a liquid and a gas standard were compared.

2.5 Determination of memory effects

Memory effects of this system were examined as follows. The standard solutions with VOCs at relatively high concentrations (50 pM each of dichloromethane, iodomethane, iodoethane, bromochloromethane, chloroform, tetrachloromethane, dibromomethane, bromodichloromethane, iodopropane, chloroiodomethane, dimethyl disulfide, dibromochloromethane, bromoform, and diiodomethane, or 500 pM of isoprene, or 50 nM of DMS) were used. The standard solution was purged, after which the solution was drained from the purging chamber and the chamber was washed with ultra pure water. After the trap column was baked at 250 °C for 15 min, the purging cham-

ber was filled with ultra pure water and the second purging was performed. The memory effect was calculated as the percentage of analytes found after the second purging in relation to the first purging.

2.6 Applications

The suitability of this system for the rapid shipboard screening and quantitative analysis of VOCs in seawater was shown through the analysis of samples collected in the open ocean.The research was carried out during a cruise of the research vessel *Mirai* during May 1999 in the northwest North Pacific Ocean, an area that exhibits a phytoplankton (for example, *Thalassiosira* spp.) bloom in the water column during this time of year. Water samples were collected at 9 depths with a CTD-rosette sampler fitted with Niskin sample bottles (General Oceanics Environmental, Inc., Miami, FL, USA). The water samples were collected in 50-mL brown glass bottles (I-CHEM Certified 200, Nalge Nunc International, Rochester, NY, USA) for the measurement of VOCs. After an overflow of more than 100 mL of water, 0.5 mL of $HgCl_2$ was added to inhibit microbial activity, and the sample bottle was immediately sealed with a three-layer septum (PTFE (polytetrafluoroethylene)/silicone/PTFE) with care to exclude air bubbles, stored in a box (in the dark), and refrigerated at 4 °C. Samples containing air bubbles were discarded. The final concentrations of $HgCl_2$ in the sample bottles were about 350 mg/L.

3 Results and discussion

3.1 Optimization of the flow rate of nitrogen

The flow rate of nitrogen purging the seawater sample to extract analytes was varied between 20 and 70 mL/min. The amount of VOCs extracted increased at higher flow rates (**Figure 2**). This effect was more remarkable for chloroiodomethane and diiodomethane than for the more volatile compounds iodomethane and isoprene. At a higher flow rate (60 mL/min) the breakthrough of more volatile compounds such as chloromethane was not observed; this breakthrough decreased the peak area of the analyte. Because an additional increase in the flow rate could not enhance the efficiency of the extraction process, the following experiments were done with a purging flow of 60 mL/min nitrogen.

3.2 Optimization of purging time

The optimization of purging time was carried out using standard solutions. The total amount of VOC purged was substantially enhanced by increasing the purging time from 5 to 20 min (**Figure 3**). Further increases of the purging time led to almost no increase in the extraction rate. For iodomethane and isoprene, the curve seemed to asymptotically reach a constant value after 20 min. Because of the lower volatility of diiodomethane, vaporization of this compound through the water surface was slower, and therefore it attained a constant value later than for the more volatile compounds. To save time and to avoid the breakthrough of analytes from the small column, a purging time of 20 min was chosen.

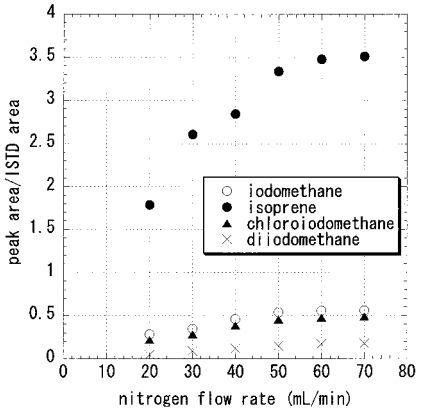

Figure 2. Optimization of the flow rate of nitrogen through the purging chamber. Purging time: 20 min. ISTD: internal standard.

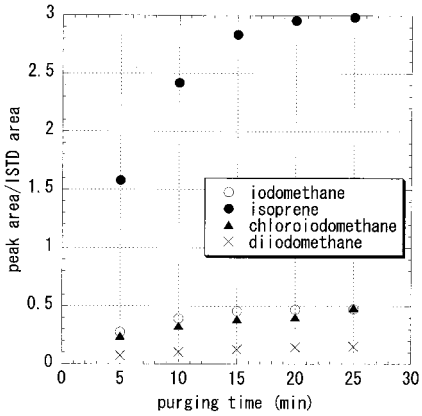

Figure 3. Optimization of purging time. Flow rate of nitrogen: 60 mL/min. ISTD: internal standard.

3.3 Optimization of trap temperature

The trap temperature was varied between −20° and 10 °C. The amount of extracted VOCs increased with decreasing trap temperature from 10° to −10 °C (**Figure 4**).This effect was most important for iodomethane and less so for diiodomethane. A further decrease in trap temperature brought no further improvement in the extraction efficiency. A general trap temperature of −10 °C was chosen.

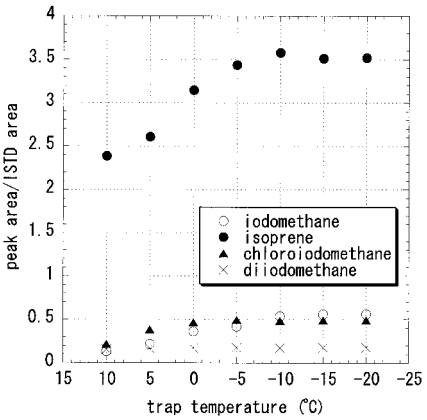

Figure 4. Optimization of trap temperature. Flow rate of nitrogen: 60 mL/min. Purging time: 20 min. ISTD: internal standard.

3.4 Comparison of two calibration plot of a liquid and a gas standard

Gas standards were introduced through a fixed 1-mL sample loop. Thus, the VOCs reached the adsorption column in a short pulse, which did not occur when a water sample was purged, when the VOCs reached the adsorption column over a longer period of time. The combined calibration curves for iodomethane and DMS, which could be prepared precisely in liquid standards as well as in gas standards, were examined to see if there was a statistically significant difference between the two types of introduction. The average difference between the two standard introduction methods was about 3%, which was within the reproducibility of the method (**Figure 5**).

3.5 Reproducibility, memory effects, linearity, and detection limits

Seven replicate measurements using 20-mL samples were reproducible to within 4.1% (**Table 3**). The memory effect observed was between 0.1 and 4.9%. Memory effects remained within an acceptable range; however, after analysis of the series of more highly contaminated samples, memory effects may have disturbed the analysis of cleaner samples. In that case, baking the trap column at 250 °C for 30 min would have diminished the memory effects. Linearity in the range 1 to 5000 pM was excellent for all VOCs. We checked the blank levels of the compounds investigated. No peak was observed. The detection limits of this system varied between 0.1 and 4.3 pM

Table 3. Relative standard deviations and detection limits by this method.

Compound	Relative standard deviation (%)	Detection limit (pM)
Chloromethane	3.8[a)]	0.9
Bromomethane	3.1[b)]	0.4
Dichloromethane	2.3[b)]	0.3
Iodomethane	2.1[b)]	0.2
Dimethyl sulfide	3.3[a)]	1.1
Iodoethane	2.5[b)]	1.0
Isoprene	1.0[b)]	0.5
Bromochloromethane	3.7[b)]	1.3
Chloroform	2.5[b)]	1.1
Tetrachloromethane	3.1[b)]	1.4
Dibromomethane	3.6[b)]	1.2
Bromodichloromethane	3.7[b)]	1.6
Iodopropane	2.6[b)]	1.8
Chloroiodomethane	2.3[b)]	1.8
Dimethyl disulfide	2.2[b)]	1.1
Dibromochloromethane	2.4[b)]	2.0
Bromoform	3.9[b)]	4.3
Diiodomethane	4.1[b)]	2.8

a) 100 pM each compound.
b) 2 pM each compound.
The detection limits were determined at a signal to noise ratio of 3 : 1.
The measurements were repeated seven times.

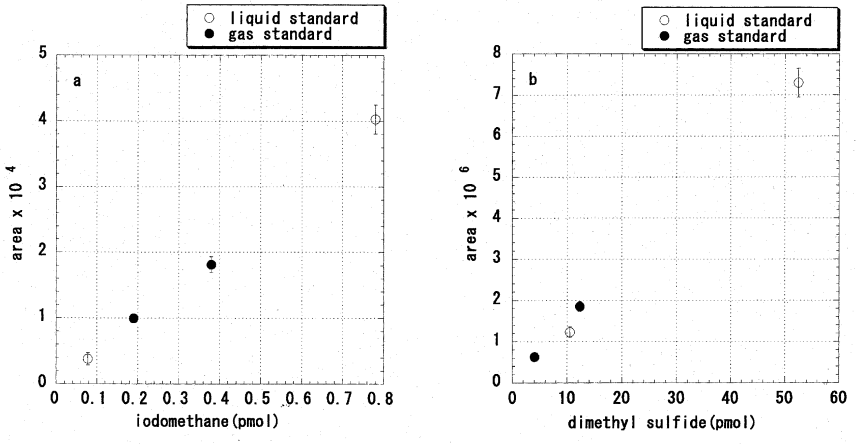

Figure 5. Combined calibration plots for liquid and gas standards. (a) iodomethane ($n = 5$), (b) dimethyl sulfide (DMS) ($n = 5$).

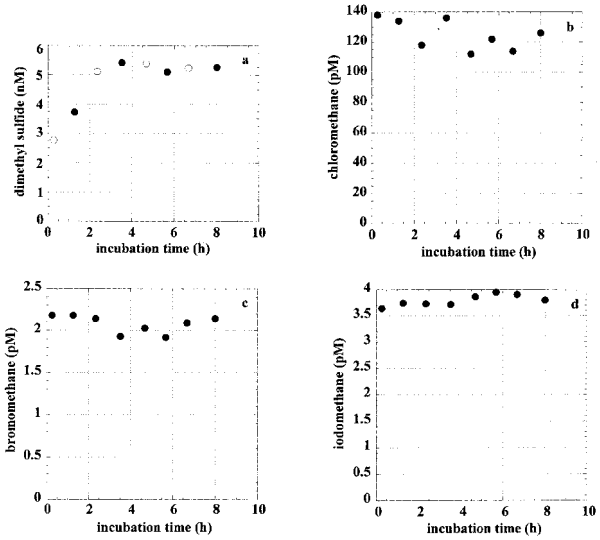

Figure 6. Time course of the concentrations of (a) dimethyl sulfide (DMS), (b) chloromethane, (c) bromomethane, and (d) iodomethane during the incubation of water samples, with (solid circles) and without (open circles) the addition of $HgCl_2$ (350 μg/L). Samples were incubated in the dark at 4 °C.

when 20-mL water samples were used, depending on the compound. The detection limits may have been limited by the volatilities of the VOCs. For volatiles with molecular weights up to that of isoprene, good detection limits of 0.2–1.1 pM were achieved with this system. We concluded that under the applied conditions, the purge and trap GC-MS system could be sensitive enough to measure VOCs in the open ocean.

3.6 Stability of the seawater sample

The stability of VOCs in stored seawater samples was evaluated by analyzing the time course of the concentrations of VOCs during the incubation of water samples, both with and without the addition of $HgCl_2$ (350 μg/L) (see **Figure 6**).

Most of the VOC concentrations in samples that were stored in the dark and refrigerated at 4 °C were stable in these experiments; however, the concentration of DMS was unstable even with addition of $HgCl_2$. This result suggested that shipboard determination was essential for the determination of unstable VOCs in seawater.

3.7 Analysis of a real sample

A real sample of seawater collected in the northwest North Pacific Ocean was analyzed. Typical chromatograms of VOCs in seawater are shown in **Figure 7**. The separation of every peak was sufficient without cryogenic focusing by liquid nitrogen.

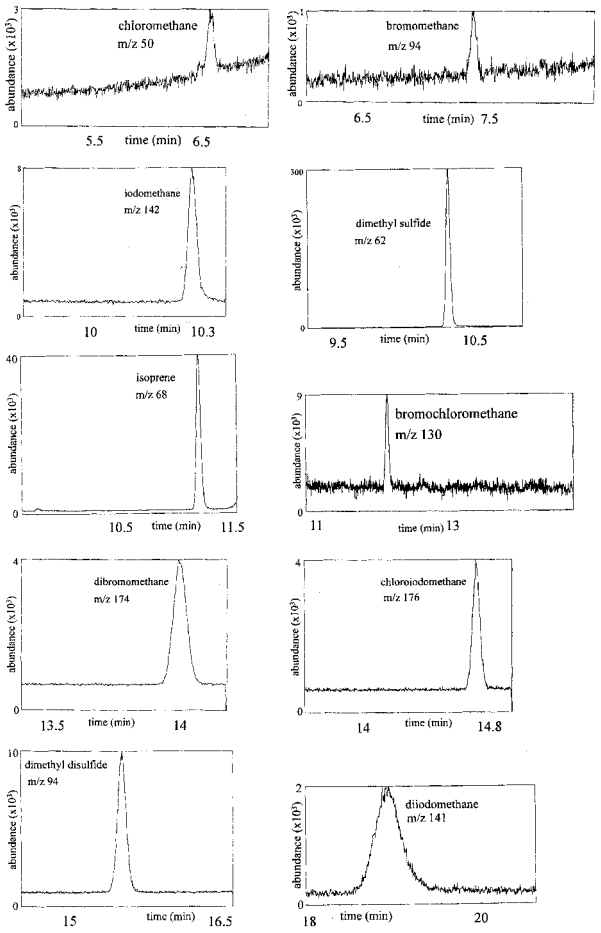

Figure 7. Ion chromatograms of a seawater sample collected in the North Pacific Ocean on May 1999.

The vertical profiles for chloromethane, bromomethane, iodomethane, and isoprene are shown in **Figure 8**. Application of the system to the analysis of seawater appeared to demonstrate the suitability of a small trap column with a purge and trap GC-MS system as a sensitive screening method for the shipboard measurement of a wide variety of VOCs.

4 Conclusion

We describe here the results of the first shipboard analysis of VOCs in seawater, with a small column coupled with an automated purge and trap GC-MS system, which would allow a better estimation of the extent of VOC emission to the atmosphere from the ocean as a whole. The data suggest that there are significant differences in DMS concentrations a few hours after sampling and, therefore, that the shipboard measurement of VOCs in the open ocean would be better than measurement at a land-based laboratory. Trace levels of VOCs in the water column in

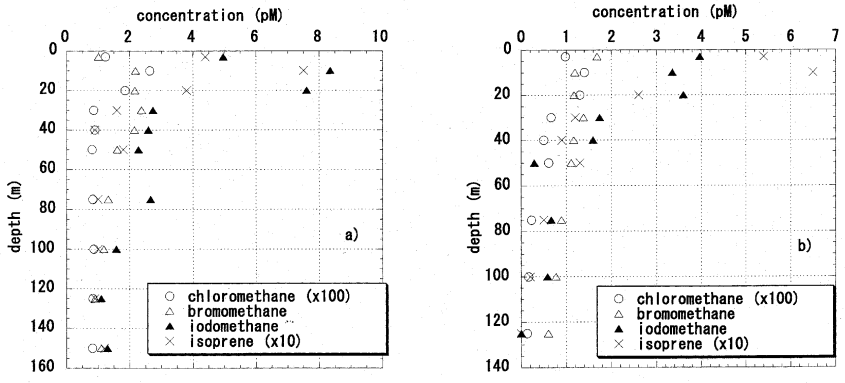

Figure 8. Vertical profiles for the North Pacific Ocean in May 1999 for chloromethane, bromomethane, iodomethane, and isoprene, plotted for the upper 100 m. Sampling date: a) 19 May, 1999, b) 22 May, 1999. Sampling location: a) 45° 50.66' N 165° 00.49' E, b) 41° 53.27' N 160° 07.04' N.

the open ocean could be determined by a fast and sensitive purge and trap GC-MS method. With this system, we were able to measure levels of these compounds in small samples (20 mL) within 1 h after sampling without cryogenic focusing. We concluded that this method could be applied for the shipboard determination of unstable VOCs in seawater.

Acknowledgment

The authors wish to express their appreciation to the captain, officers, and crew of the R.V. *Mirai* for their excellent service.

References

[1] R.J. Charlson, J.E. Lovelock, M.O. Andreae, S.G. Warren, *Nature* **1987**, *326*, 655.

[2] R.M. Moore, R. Tokarczyk, *Global Biogeochem. Cycles* **1993**, *7*, 195–210.

[3] R.R. Dickerson, K.P. Rhoads, T.P. Carsey, S.J. Oltmans, J.P. Burrows, P.J. Crutzen, *J. Geophys. Res.* **1999**, *104*, 21385–21395.

[4] W.J. De Bruyn, T.S. Bates, J.M. Cainey, E.S. Saltzman, *J. Geophys. Res.* **1998**, *103*, 16703–16711.

[5] P.D. Nightingale, G. Malin, P.S. Liss, *Limnol. Oceanogr.* **1995**, *40*, 680–690.

[6] V.K. Tait, R.M. Moore, *Limnol. Oceanogr.* **1995**, *40*, 189–195.

[7] S.L. Manley, J.L. de la Cuesta, *Limnol. Oceanogr.* **1997**, *42*, 142–147.

[8] S. Sæmundsdóttir, P.A. Matrai, *Limnol. Oceanogr.* **1998**, *43*, 81–87.

[9] R.M. Moore, *Chemosphere: Global Change Science* **2000**, *2*, 95–99.

[10] Y. Yokouchi, H. Bandow, H. Akimoto, *J. Chromatogr.* **1993**, *642*, 401–407.

[11] M.P. Maskarinec, L.H. Johnson, S.K. Holladay, R.L. Moody, C.K. Bayne, R.A. Jenkins, *Environ. Sci. Technol.* **1990**, *24*, 1665–1670.

[12] R. Simó, A.D. Hatton, G. Malin, P.S. Liss, *Mar. Ecol. Prog. Ser.* **1998**, *167*, 291–296.

[13] J.W.H. Dacey, S.G. Wakeham, *Science* **1986**, *233*, 1314–1316.

[14] D. Price, R.F.C. Mantoura, P.J. Worsfold, *Anal. Chim. Acta* **1998**, *377*, 145–155.

[15] J.E. Johnson, *Anal. Chim. Acta* **1999**, *395*, 119–132.

[16] D.C. Lowe, W. Allan, M.R. Manning, T. Bromley, G. Brailsford, D. Ferretti, A. Gomez, R. Knobben, R. Martin, Z. Mei, R. Moss, K. Koshy, M. Maata, *J. Geophys. Res.* **1999**, *104*, 26125–26135.

[17] A.M. Featherstone, P.R. Boult, B.V. O'Grady, E.C.V. Butler, *Anal. Chim. Acta* **2000**, *409*, 215–226.

[18] M.D. DeGrandpre, T.R. Hammar, C.D. Wirick, *Deep-Sea Res.* **1998**, *45*, 1557–1575.

[19] T. Hino, S. Nakanishi, T. Maeda, T. Hobo, *J. Chromatogr. A* **1998**, *810*, 141–147.

[20] S. Hashimoto, H.Y. Sun, T. Nakamura, Y. Nojiri, A. Otsuki, *Geochem. J.* **1993**, *27*, 117–123.

[21] C. Schall, K.G. Heumann, *Fresenius J. Anal. Chem.* **1993**, *346*, 717–722.

[22] S. Hashimoto, Y. Kurita, Y. Takasu, A. Otsuki, *Deep-Sea Res.* **1998**, *45*, 301–316.

[23] W. Groszko, R.M. Moore, *Chemosphere* **1998**, *36*, 3083–3092.