

# Determination of Concentration and Carbon Isotopic Composition of Dissolved Methane in Sediments and Nearshore Waters

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Techniques have been developed that allow on-line simultaneous analysis of concentration and carbon isotopic composition of dissolved methane in seawater and porewater using isotope-ratio-monitoring gas chromatography/mass spectrometry. The method uses either headspace equilibration or He-sparging of water, followed by sample drying, cryofocusing, subambient GC separation using a PoraPLOT-Q analytical capillary column, on-line 1000 or 1150 °C combustion, and measurement on a MAT 252 isotope-ratio-monitoring mass spectrometer. Analyses of porewaters using headspace equilibration take less than 15 min per sample whereas the He-sparging technique requires ~30 min/sample. The detection limit with an isotopic ratio standard deviation of 0.5‰ is 10 nM using 10 mL samples. Analytical blanks associated with these methods are negligible. The procedures were evaluated through analyses of porewater CH<sub>4</sub> in a core taken in muddy sediments of Tomales Bay, CA, and of interstitial and surface seawater from Checker Reef in Kaneohe Bay, Oahu, HI. Carbon isotopic analyses of low concentrations of dissolved CH<sub>4</sub> in porewaters should prove to be a useful monitor of anaerobic diagenesis of sedimentary organic matter and the origins of CH<sub>4</sub>, especially in systems where inputs of organic matter are low.

Organic matter decomposition in sediments is an important process in global and local carbon budgets as it ultimately recycles complex organic compounds from terrestrial and aquatic environments to carbon dioxide and methane. Organic matter decomposition in oxic sediments is largely accomplished by individual organisms that can oxidize complex organic compounds completely to CO<sub>2</sub>. In contrast, organic matter decomposition in anoxic sediments requires integrated microbial communities containing fermenting and respiring organisms that interact by the exchange of a variety of organic and inorganic intermediates (e.g., ref 1). Methane is a major component in the carbon cycle of anaerobic aquatic systems, particularly those with low sulfate concentrations. Carbon isotopic compositions have provided important information on sources and fates of CH<sub>4</sub> in such

systems.<sup>2-4</sup> Unfortunately, conventional carbon isotopic analyses of CH<sub>4</sub> require enough material to prepare micromole quantities of CO<sub>2</sub>, and this limitation has restricted isotopic analyses to sedimentary environments particularly rich in CH<sub>4</sub>.

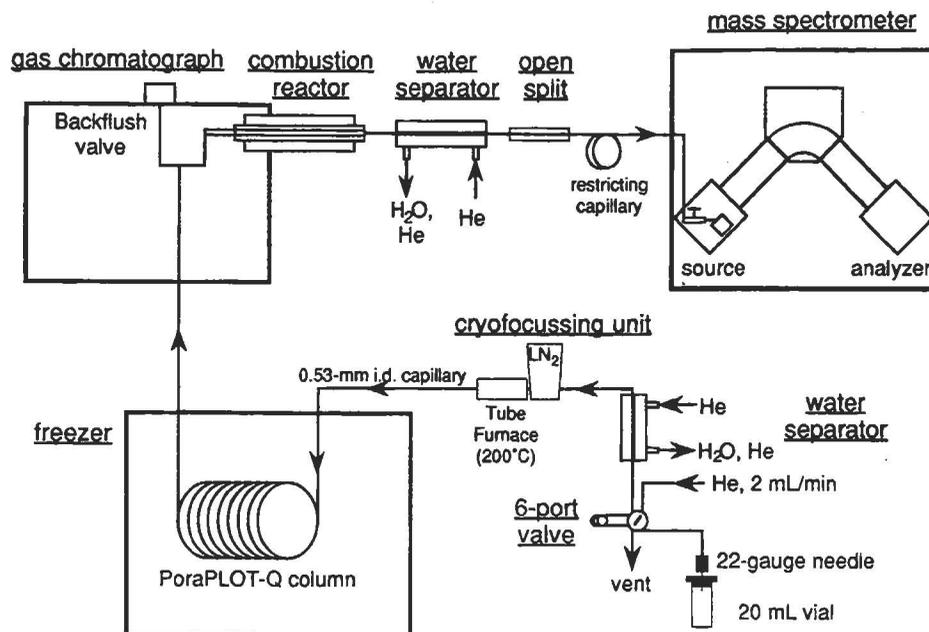
Isotope-ratio-monitoring gas chromatography/mass spectrometry (irm-GC/MS) systems can reduce drastically the size of sample needed for isotopic analyses.<sup>5-7</sup> These systems quantitatively convert gas chromatographic effluents to CO<sub>2</sub>, remove water of combustion, and deliver the CO<sub>2</sub> continuously to the ion source of the isotope-ratio mass spectrometer using helium carrier gas. These systems thereby increase the speed and decrease the amount of sample needed for analyses. Recent reports have documented the ability of irm-GC/MS systems to generate accurate and precise isotopic data for a variety of samples including geolipids,<sup>6,8,9</sup> anaerobic incubator headspace gases,<sup>10,11</sup> and synthetic and atmospheric CH<sub>4</sub>.<sup>12</sup> The work presented in this article builds upon this previous research and applies irm-GC/MS techniques to the study of trace quantities of dissolved CH<sub>4</sub> in natural waters.

This paper describes two procedures we developed to analyze simultaneously the concentration and carbon isotopic composition of dissolved CH<sub>4</sub>. The first method is useful for analyses of dissolved CH<sub>4</sub> in porewater of fine-grained sediments where water volume is limited. As part of a larger study of the carbon budget of an estuary,<sup>13</sup> the procedure developed was used to study the origins and fate of sedimentary CH<sub>4</sub>. The second procedure is useful where CH<sub>4</sub> concentrations are very low, but where water volume is large. The method was evaluated by comparing results

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**Figure 1.** Schematic diagram of the system used for  $\text{CH}_4$  headspace sampling, cryogenic focusing, subambient gas separation, on-line combustion, and isotopic analysis by isotope-ratio-monitoring mass spectrometry. See text for details.

of isotopic analyses of reef interstitial water using this method with results previously obtained using conventional techniques.<sup>14,15</sup>

## EXPERIMENTAL SECTION

### Headspace Analyses. (a) Collection of Sediment Samples.

Samples for headspace analysis were obtained in March 1993 from a 3.3 m sediment core taken in Tomales Bay, CA, at station 16 (see ref 13 for location and site description). A recently developed piston coring technique was used to collect the sediment core.<sup>16</sup> Methane was collected using a modification of the whole-sediment headspace equilibration technique of Alperin and Reeburgh.<sup>17</sup> Briefly, at least two subcores of sediment were taken at 20 cm intervals, using either 2.5 or 1.0 mL tipless glass syringes, and transferred to a 20 mL glass crimp-top serum vial. The serum vial contained 1.0 mL of He-sparged saturated  $\text{HgCl}_2$  solution as a preservative as well as 2.5 mL of He-sparged deionized (DI)  $\text{H}_2\text{O}$  and was flushed with He for 1 min prior to addition of sediment. The vial was sealed with a gray butyl rubber stopper and the sediment homogenized by vortex mixing for >60 s. Samples were kept in cool, dry locations throughout shipment to Hawaii and storage. Methane concentrations were obtained separately during processing of sediment cores at intervals where material was collected for isotopic analyses. An aliquot of the headspace was quantitated using a HP 8290 gas chromatograph equipped with a flame ionization detector and a stainless steel packed column (Porapak-Q,  $\frac{1}{4}$  in.  $\times$  3 m).

**(b) Analytical System.** A  $\sim$ 6 cm, 22 gauge needle (removed from its Luer-lock hub) was attached to a zero dead volume Valco

six-port injection valve ( $\frac{1}{16}$  in. port diameter, Hastalloy C) using stainless steel capillary tubing and a Swagelok union (Figure 1). Depending on the concentration of dissolved  $\text{CH}_4$ , a stainless steel sample loop ranging in size from 15  $\mu\text{L}$  to 2 mL was attached to the six-port valve. Water vapor was removed by passing the injected headspace gas through a 20 cm length of Nafion tubing (Perma Pure Inc., Toms River, NJ) with a 10 mL/min counterflow of helium. Dry headspace gases were cryofocused by transfer through a 0.53 mm i.d. capillary column (Hewlett-Packard Ultra 1) and absorption at liquid nitrogen temperatures onto a 2 cm long Porapak-Q (80–120 mesh) segment that had been manually packed (Figure 2). Liquid nitrogen was contained in a Styrofoam cup perforated with a 10 cm length of Teflon tubing through which the capillary column containing the plug of Porapak-Q could slide. Gases were desorbed from the Porapak-Q using a minitube furnace fabricated by using 6 mm o.d. quartz tubing wrapped with  $\sim$ 1 m of 24 gauge Ni-chrome wire and maintained at 200  $^\circ\text{C}$  with an Omega CN9000A temperature controller (Omega Engineering Inc., Stamford, CT).  $\text{CH}_4$  was separated from  $\text{N}_2$  and  $\text{CO}_2$  by use of a 25 m  $\times$  0.32 mm PorapLOT-Q (Chrompack Inc., Raritan, NJ) analytical capillary column at a flow rate of 2 mL/min at subambient temperatures, generally  $-25$   $^\circ\text{C}$ .

Samples were combusted, water from combustion was removed, and isotopic composition was determined using a MAT 252 irm-GC/MS (Finnigan MAT, Bremen, Germany) system previously described by Hayes et al.<sup>6</sup> The only modification to this system was the temperature of combustion, which was held constant at 1000  $^\circ\text{C}$  when CuO/Pt was used as an oxidant. Monitoring mass 16 ( $\text{CH}_4$ ) and 28 (CO) during analyses of a  $\text{CH}_4$  laboratory standard indicated complete oxidation of  $\text{CH}_4$  to  $\text{CO}_2$  under these conditions (see also ref 12). However, combustion of  $\text{CH}_4$  rapidly reduces CuO, which results in decreased combustion efficiency.<sup>18,19</sup> Excellent combustion efficiency and extended

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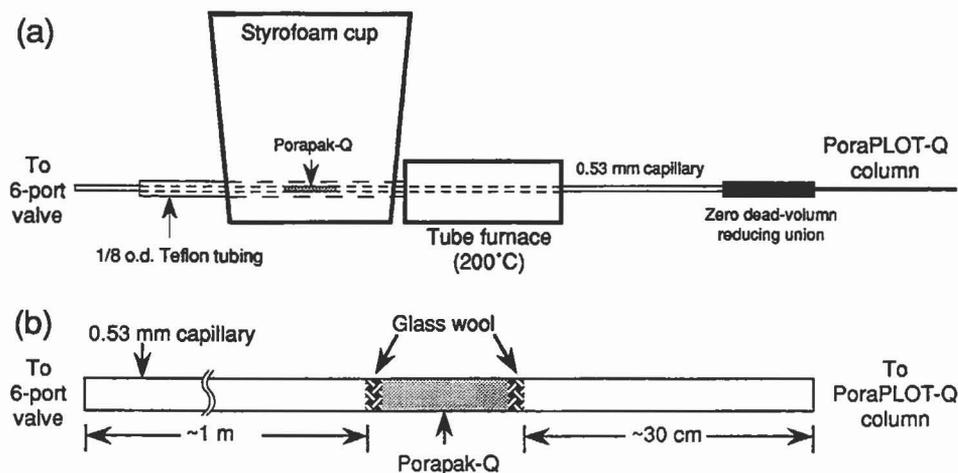
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**Figure 2.** Schematic diagram of cryofocusing apparatus. (a) Illustration of construction of liquid nitrogen container and tube furnace. Gases are adsorbed onto Porapak-Q when the plug is immersed in liquid nitrogen, as shown. To desorb the gases rapidly, the plug is manually moved into the tube furnace. (b) Detail of 0.53 mm i.d. capillary packed with a segment of Porapak-Q (80–120 mesh). Porapak-Q is packed manually and held in place between small plugs of glass wool. See text for details.

life was achieved by replacing the CuO/Pt with NiO<sub>2</sub>/Pt and maintaining the combustion temperature at 1150 °C.<sup>12,18,19</sup> The open split on the mass spectrometer was operated at a 4:1 ratio.

**(c) Analytical Procedures.** Transfer of headspace gases to the sample loop was accomplished by pressurizing the headspace of the serum vial by addition of He-sparged DI H<sub>2</sub>O and then puncturing the septum of the pressurized vial, allowing headspace gases to flow into the sample loop.

Isotopic analysis by irm-GC/MS of organic compounds requires transfer of CO<sub>2</sub> from combustion as a finely focused peak into the mass spectrometer. Transfer of gases from the sample loop to the cryofocusing segment was complete within 2–4 min, depending on the size of the sample loop. Rapidly heating the plug of Porapak-Q to 200 °C released the gases into a narrow band in the capillary column. Without cryofocusing, low signal/noise ratios were obtained, which rendered the analysis useless.

A high concentration of N<sub>2</sub> in porewater produced tailing of the N<sub>2</sub> peak, which caused a pressure pulse in the ion source of the mass spectrometer and interfered with the CH<sub>4</sub> peak. To avoid interference of N<sub>2</sub> with CH<sub>4</sub>, the N<sub>2</sub> peak was vented to atmosphere by “back-flushing” during elution of N<sub>2</sub> (Figure 1). Typically, back-flushing for the first 170 s of the analysis was sufficient to eliminate interference from N<sub>2</sub> when the column was held at –25 °C.

Standardization was achieved using techniques outlined by Merritt et al.<sup>20</sup> Briefly, isotopic standards were introduced by admitting pure CO<sub>2</sub> from a gas stream through a second capillary leak attached to the ion source. Although standards can be introduced at nearly any time during a chromatographic analysis, standards were introduced to bracket elution of the CH<sub>4</sub> peak.  $\delta$  values were determined by comparing background-corrected mass ratios of the pure CO<sub>2</sub> gas with that of the sample.<sup>20</sup> All isotopic ratios are expressed in standard  $\delta$  notation in per mil (‰) relative to the PDB standard.

Collection and processing of isotope ratio results are described in detail by Ricci et al.<sup>21</sup> Briefly, the MAT 252 mass spectrometer was computer controlled and data acquisition events were under

software control. Three ion currents were measured simultaneously for CO<sub>2</sub> species at  $m/z$  44, 45, and 46. Signals were digitized and stored at 0.25 s intervals, the data files were scanned using a procedure that separated sample from standard signals, and conventional techniques were used to calculate  $\delta$  values.<sup>21</sup> Using these procedures, peak areas of  $m/z$  44, 45, and 46 were determined, which allowed us to quantitate CH<sub>4</sub> from the peak area of the major ion ( $m/z$  44).

**Gas Sparging Analyses. (a) Collection of Reef Interstitial and Surface Seawater.** Interstitial seawater was collected from wells in Checker Reef, Kaneohe Bay, Oahu, HI, by use of well points (see refs 22 and 23 for details of sampling and site description). For isotopic analyses of CH<sub>4</sub>, reef interstitial water was pumped directly into 40 mL serum vials containing a Teflon-coated magnetic stir bar, 2 mL of saturated HgCl<sub>2</sub> solution was added as a preservative, and the vial was sealed with a gray butyl rubber stopper. Approximately 125 mL of surface seawater from the windward margin of the forereef was similarly collected and preserved. Samples of porewater and surface seawater were collected for determination of concentration and isotopic composition of total dissolved inorganic carbon (DIC). These samples were stored in 20 mL serum vials with 1 mL of saturated HgCl<sub>2</sub> solution added, and the vial sealed with a gray butyl rubber stopper. Samples were prepared for isotopic analyses of DIC using standard methods.<sup>24</sup>

**(b) Analytical System.** Helium for sparging was admitted to the sample through a ~4 cm long, 22 gauge needle (removed from its Luer-lock hub) attached to 1/16 in. stainless steel capillary tubing using a Swagelok union (Figure 3). The flow of helium and sparged gases from the sample passed through a ~6 cm long, 22 gauge needle attached by a Swagelok union to 1/16 in. stainless steel capillary tubing. Approximately 2 mL of water was displaced during sparging and was trapped by passing it into a 6 mm o.d. glass capillary column with a ~10 mL bulb. Headspace gases were dried using a 40 cm length of Nafion tubing with a 20 mL/

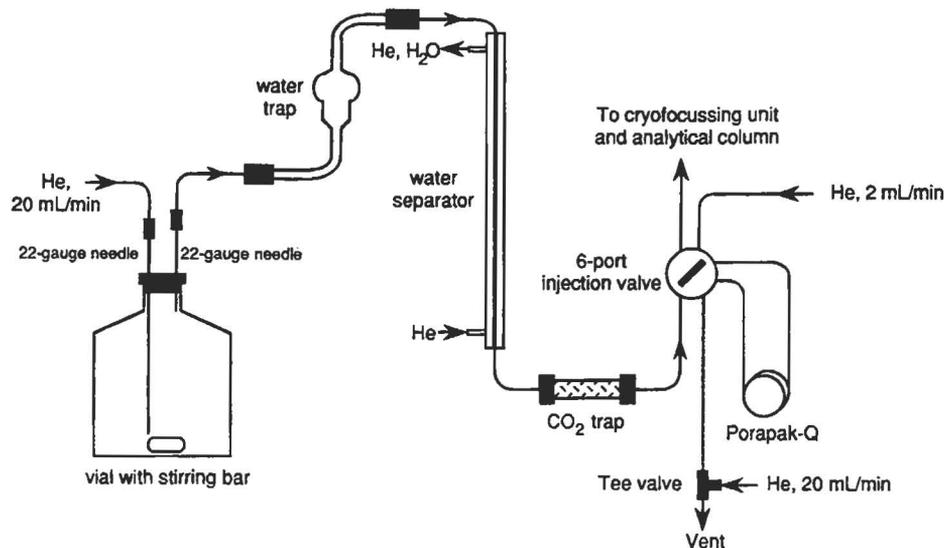
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**Figure 3.** Schematic diagram of system used for sparging  $\text{CH}_4$  from waters, drying gases, separating  $\text{CO}_2$ , and trapping the remaining gases. See text for details.

min counterflow of helium. Carbon dioxide was removed from the sparged gases using a 5 cm long (3 mm i.d.) column packed with Ascarite II (Thomas Scientific, Swedesboro, NJ). The remaining sparged gases were trapped at liquid nitrogen temperature on a 15 cm long, 3.2 mm o.d. stainless steel column packed with Porapak-Q (80–120 mesh) attached as the sample loop to the six-port Valco injection valve. The vent was connected to a tee valve, which allowed helium purging and flushing of transferred water from the system after the sparging of each sample was completed. Separation and analysis of  $\text{CH}_4$ , including cryofocusing and chromatography, was identical to that described above for the headspace gases.

**(c) Analytical Procedures.** Most water samples analyzed required  $\sim 10$  mL for the analysis. For these samples, an empty 20 mL glass vial containing a Teflon-coated magnetic stir bar was capped with a gray butyl rubber septum, the septum punctured with the needles described above, and the vial flushed with helium for 5 min. Porewater was removed from the 40 mL vials by displacement with He-sparged DI water by using gas-tight syringes. The Porapak-Q column was cooled to liquid nitrogen temperature, and water slowly added to the 20 mL vial. Sparging for 15 min was sufficient to quantitatively transfer  $\text{CH}_4$  to the Porapak-Q packed column. Sparging efficiency was improved dramatically when samples were stirred.

Atmospheric contamination was minimized by purging all lines with helium for at least 30 s prior to puncturing the septum with the needles. However, to avoid overpressurization of the sample vial, the flow of helium in the transfer line to the trap must be reversed at the tee fitting prior to perforation of the septum. Inserting the needles partially into the septum allowed ample time to reverse the flow yet kept contamination to a minimum.

As a result of the large internal volume of the Porapak-Q column, transfer of gases to the cryofocusing segment required at least 6 min. Gases were desorbed from the Porapak-Q trap by immersing the column in boiling water. Combustion, peak quantitation, and determination of isotopic ratios was as described above.

## RESULTS AND DISCUSSION

**Precision and Accuracy of Analyses.** Results of  $m/z$  44 peak area calibration using direct injection of varying volumes of

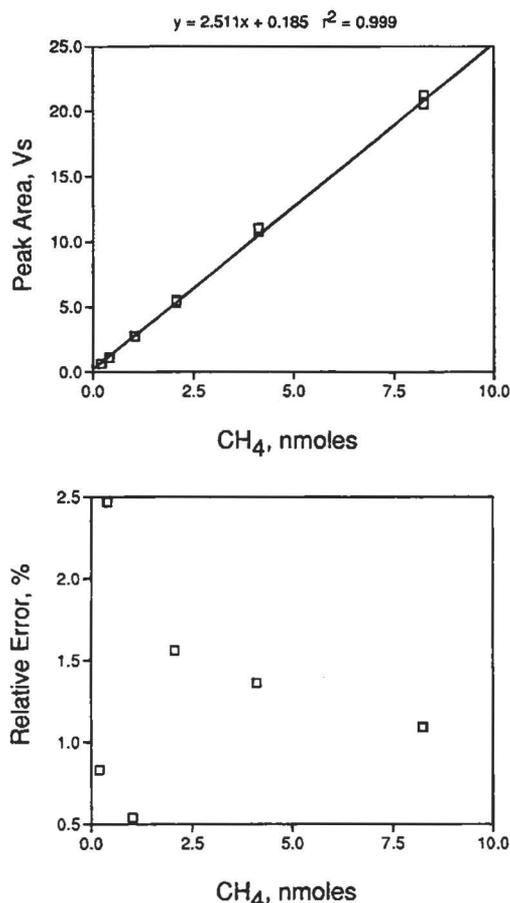
a 99.18 ppm  $\text{CH}_4$  standard are shown in Figure 4a. Relative error in concentration determinations, using samples ranging from 0.2–8.3 nmol of  $\text{CH}_4$ , was less than 3% (Figure 4b). However, variation in peak area from day to day was found to be as high as 30%, thus necessitating daily calibration. Daily calibration was sufficient to maintain constant and small relative error in concentration determinations ( $<3\%$ ) during  $\sim 8$  h work days. The sensitivity of this method can be increased by reducing the split ratio.

The accuracy of concentration determinations of gas-sparged water samples was determined by comparison of concentrations determined using the irm-GC/MS technique with the gas-sparging GC technique of Brooks et al.<sup>25</sup> Results of these analyses, which show good agreement, are shown in Figure 5. Results of isotopic analyses show that differences between duplicate samples of concentrations greater than 35 nM are typically less than 0.5% (Table 1). The greater variation in the surface seawater samples may have resulted from inadequate sparging of  $\text{CH}_4$  or from an analytical blank (see below).

Results of isotopic analyses of these samples as a function of sample size are shown in Figure 6a. Accuracy of isotopic analyses was found to be less than 0.3% vs PDB using procedures described by Merritt et al.<sup>12</sup> with a  $\text{CH}_4$  standard for which the isotopic composition was determined by conventional methods. Precision, as calculated from the standard deviation of the mean, varied as a function of the size of sample analyzed (Figure 6b). For injections greater than 1 nmol of  $\text{CH}_4$ , the standard deviation of the mean was less than 0.25%. Results of isotopic analyses of gas samples containing 0.7–1.4 nmol of  $\text{CH}_4$  (calculated by use of a 4:1 split ratio) fall short within a factor of 10 of the theoretical performance of this instrument.<sup>7</sup> We suspect that this loss of performance resulted from sample handling and the relatively large split ratio. Such results are also characteristic of the performance now being obtained with irm-GC/MS systems for analyses of more complex hydrocarbons.<sup>6,8,9</sup> We have found these results to be representative of the long-term ( $\sim 1.5$  year) performance of the system.

**Evaluation of Analytical Blanks.** Analytical blanks for carbon isotopic analyses of organic materials can be evaluated

(25) Brooks, J. M.; Reid, D. F.; Bernard, B. B. *J. Geophys. Res.* **1981**, *86*, 11029–11040.

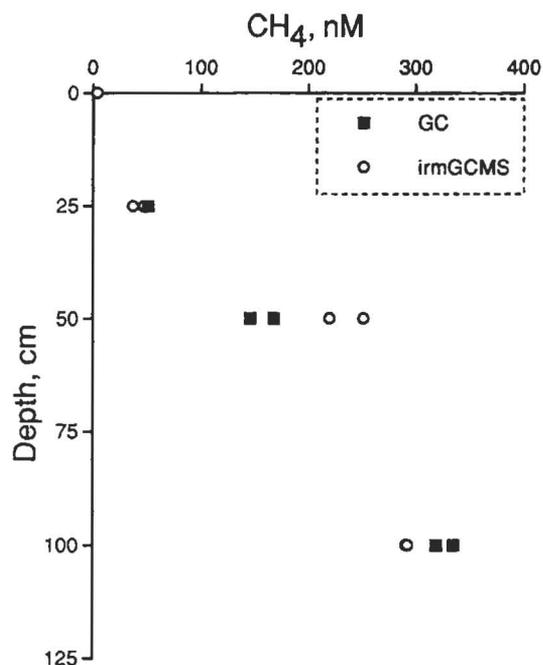


**Figure 4.** Sample size plotted as a function of peak area and relative error. The concentration of  $\text{CH}_4$  shown is that in the sample loop and not that of the resulting  $\text{CO}_2$  that entered the mass spectrometer. (a) Size of sample was varied by using different size sample loops (15  $\mu\text{L}$  to 2 mL). Peak start and stop were taken at rates of 0.10 V/s, and the peak was integrated every 0.25 s. Results of type I regression analysis are shown. (b) Size of sample plotted as a function of relative error of four to eight replicate analyses at each concentration, where the relative error was taken as (standard deviation/mean)  $\times$  100.

through regression of the isotopic composition of the sample onto the inverse of the sample size.<sup>7,26</sup> For samples of a 99.18 ppm  $\text{CH}_4$  standard, we found no systematic inaccuracies in the isotopic composition of  $\text{CH}_4$  as a function of sample size, indicating that effects of analytical blanks on isotopic compositions were minimal (Figure 6a). The effects of analytical blanks on samples of headspace gases can be evaluated from the results of analyses of multiple samples of varying size (2–5 g, wet weight) taken at the same horizon in the sediment core (207 cm). Again, no systematic relationship between sample size and isotopic composition was observed in the sediment samples, indicating the effects of the analytical blanks were minimal (Figure 7). In addition, vials containing only He-sparged  $\text{HgCl}_2$ -saturated  $\text{H}_2\text{O}$  processed in parallel with samples had no detectable  $\text{CH}_4$ .

To determine whether analytical blanks affected the isotopic composition of  $\text{CH}_4$  sparged from water, various volumes of 99.18 ppm  $\text{CH}_4$  were added to degassed seawater using a gas-tight syringe and the gases sparged, trapped, and analyzed. Prior to these analyses, the  $m/z$  44 peak area was quantitated by direct injection of the 99.18 ppm standard using methods described

(26) Gelwicks, J. T.; Hayes, J. M. *Anal. Chem.* 1990, 62, 535–539.



**Figure 5.** Comparison of concentration of  $\text{CH}_4$  in reef interstitial water determined using GC gas stripping and irm-GC/MS techniques. Replicate samples for GC and irm-GC/MS analyses were obtained from the same well at the time of sampling.

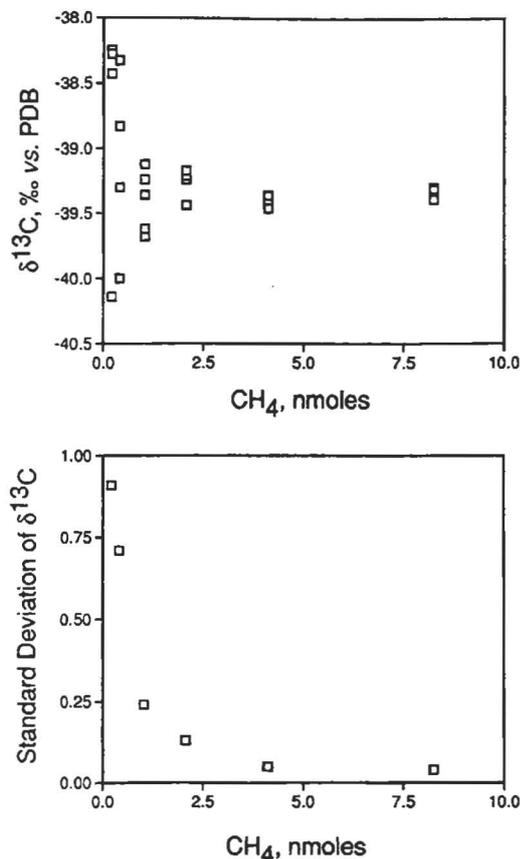
**Table 1. Results of Concentration and Isotopic Analyses of Reef Interstitial Water<sup>a</sup>**

| depth, cm | vol analyzed, mL | $[\text{CH}_4]$ , nM | $\delta^{13}\text{C}$ , ‰ vs PDB |
|-----------|------------------|----------------------|----------------------------------|
| 0         | 160.20           | 4                    | -54.5                            |
| 0         | 157.69           | 4                    | -56.6                            |
| 25        | 59.79            | 37                   | -51.1                            |
| 25        | 59.11            | 47                   | -51.0                            |
| 50        | 10.00            | 220                  | -50.6                            |
| 50        | 10.00            | 252                  | -50.4                            |
| 100       | 5.00             | 293                  | -51.2                            |
| 100       | 10.00            | 273                  | -51.0                            |

<sup>a</sup> Duplicate samples were taken for irm-GC/MS analyses from the same well. Analyses of samples at 50 and 100 cm were on 10 mL of water removed by displacement with He-sparged water. Two 10 mL aliquots were removed from the samples at 50 cm and analyzed separately.

above, thus allowing calculation of the size of the standard injected into the degassed seawater plus the analytical blank. The analytical blank determined in this manner was  $20 \pm 60$  pmol of  $\text{CH}_4$ . The isotopic composition of the analytical blank determined using the method of Gelwicks and Hayes<sup>26</sup> was  $-19 \pm 15\%$ . The uncertainty in this value is largely due to uncertainty in the magnitude of the analytical blank. We suspect that the analytical blank resulted from trace quantities of  $\text{CH}_4$  in the helium used to sparge the seawater samples. The analytical blank may be reduced by first passing the helium used for sparging through a Porapak-Q trap held at liquid nitrogen temperatures.

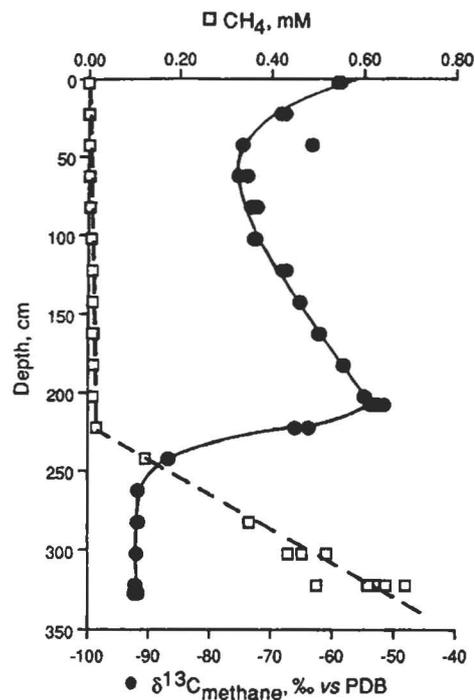
**Evaluation of Methods.** The procedures were evaluated through analyses of porewater  $\text{CH}_4$  in a sediment core taken in Tomales Bay, CA (Figure 7) and of porewater and surface seawater from Checker Reef in Kaneohe Bay, Oahu, HI (Table 1). Reflecting the depth of sulfate depletion in Tomales Bay sediments, significant  $\text{CH}_4$  accumulation begins at a depth of 240 cm. Minima in the carbon isotopic composition of  $\text{CH}_4$  ( $< -85\%$ ) occur just below the zone of sulfate reduction and at the base of



**Figure 6.** Carbon isotopic composition of a  $\text{CH}_4$  standard and standard deviation of analyses plotted as a function of sample size. (a) Size of sample was varied as described in Figure 4. (b) Standard deviation represents one standard deviation about the mean of four to eight analyses.

the zone of bioturbation. These minima reflect production of  $\text{CH}_4$ , in the latter case probably within anoxic microenvironments. Maxima in  $\delta^{13}\text{C}$   $\text{CH}_4$  occur at the sediment surface and at the base of the zone of sulfate reduction. Enrichment of  $^{13}\text{C}$  in  $\text{CH}_4$  at the surface probably reflects aerobic  $\text{CH}_4$  oxidation (or possibly, fractionation due to  $\text{CH}_4$  diffusion upwards), whereas  $^{13}\text{C}$  enrichment at depth most likely results from anaerobic oxidation.<sup>3</sup> Carbon isotopic compositions of Checker Reef porewater average  $-50\text{‰}$ , show little variation with depth (25–200 cm), and are enriched in  $^{13}\text{C}$  relative to surface seawater. Our results for interstitial waters are generally consistent with those obtained using conventional methods.<sup>14,15</sup> Isotopic compositions of these porewaters probably reflect aerobic  $\text{CH}_4$  oxidation, whereas isotopic composition of surface seawater is likely controlled by inland sources of  $\text{CH}_4$ . This interpretation is supported by results of isotopic analyses of porewater DIC. These results show  $\sim 2\text{--}3\text{‰}$  depletion in  $^{13}\text{C}$  relative to the overlying seawater, indicating either rapid mixing with overlying seawater or low rates of respiration. Results of analyses of  $\text{CH}_4$  and porewater DIC in the coral reef framework confirm earlier results indicating that  $\text{CH}_4$  production occurring in microzones is coupled with oxidation within a widespread suboxic zone in the upper meter of the reef.<sup>23</sup>

**Comparison with Other Methods.** A variety of methods has been used to characterize the isotopic composition of sedimentary  $\text{CH}_4$ . Many techniques rely on release of  $\text{CH}_4$  through bubble ebullition during stirring of sediment, which provides only a sample of homogenized  $\text{CH}_4$  from the uppermost ( $\sim 10\text{--}30$  cm) sedi-



**Figure 7.** Concentration and carbon isotopic composition of dissolved  $\text{CH}_4$  in porewater plotted as a function of depth in sediments of Tomales Bay, CA. Isotopic composition of  $\text{CH}_4$  was determined using the irm-GC/MS technique described in the text, and concentration shown was determined using the method of Alperin and Reeburgh<sup>17</sup> as described in the text. The placement of lines shown in this figure is subjective and is only intended to represent the principal trends of the data. Note the replication of six subcores taken from a depth of 207 cm with sediment samples ranging in size from 1.0 to 4.3 g (wet weight). Standard deviations about the mean of six analyses was 0.9‰. The greater variation found for sediment samples compared to the  $\text{CH}_4$  standard is likely due to heterogeneity in samples and loss of gas during transfer of mud.

ment.<sup>27,28</sup> Techniques for extraction and isotopic analyses of  $\text{CH}_4$  from unconsolidated sediments have been used to yield profiles of  $\delta^{13}\text{C}$   $\text{CH}_4$ .<sup>3,29</sup> However, these methods require  $\sim 100$  cm<sup>3</sup> of wet sediment ( $\sim 225$  g) and more than 45 min/sample preparation time. Furthermore, they are limited to sediments yielding more than  $5$   $\mu\text{mol}$  of  $\text{CH}_4$  to obtain a precision of  $\pm 0.1\text{‰}$ .<sup>3,29</sup> Methane prepared using these methods is susceptible to contamination by carbon compounds other than  $\text{CH}_4$  that have significant vapor pressure at liquid nitrogen temperatures (e.g., carbon monoxide, ethane) and to insufficient trapping of  $\text{CO}_2$ .<sup>3,29</sup> A similar method was used to determine the carbon isotopic composition of dissolved  $\text{CH}_4$  in reef interstitial water.<sup>14,15</sup> This method required  $\sim 2.5$  L of interstitial water and was subject to the same technical limitations as the other sediment methods.<sup>3,29</sup> In comparison, our techniques produce isotopic measurements of similar accuracy and precision but with smaller samples containing lower concentrations of  $\text{CH}_4$ . In addition, our methods require significantly less time and are not subject to contamination from other carbon compounds.

## CONCLUSIONS

A method has been developed that allows simultaneous measurement of concentration and isotopic composition of trace

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quantities of dissolved  $\text{CH}_4$  in natural waters. The method is rapid, taking from 15 to 30 min from sample injection to analysis, and utilizes equipment commercially available. Although we used this technique for analyses of dissolved  $\text{CH}_4$  in marine systems, it should be amenable with little modification to isotopic analyses of  $\text{CH}_4$  dissolved in other natural waters with similar concentrations (e.g., lake or river water). The technique yielded accurate and precise isotopic data on samples containing as little as 200 pmol of  $\text{CH}_4$ . Effects of analytical blanks appear to be minimal.

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#### Addendum to Popp *et al.* (1995) and Sansone *et al.* (1997):

We have recently modified the procedures described in these papers as follows: the liquid nitrogen in the cooling bath for the stainless steel Porapak-Q cold trap has been replaced by a liquid-nitrogen/ethanol slush (-118°C). This decreases the amount of  $\text{N}_2$  retained by the cold trap and ensures better separation of the  $\text{CH}_4$  peak from the  $\text{N}_2$  peak during the subsequent PLOT chromatography.