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Stable Carbon Isotopic Analysis of Low-Level Methane in Water and Gas

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Sample introduction systems have been developed to significantly extend existing isotope ratio monitoring gas chromatography/mass spectrometry techniques to allow the simultaneous determination of the concentration and stable carbon isotopic ratio of methane from low-concentration aqueous and gaseous samples. These systems, along with improvements to the basic analytical system, are used with 1−50 nM water samples and 45 ppb−250 ppm (v/v) gas samples. The detection limit of the technique is 200 pmol of CH_4 in either aqueous or gaseous samples. The precision of isotopic measurements of replicate samples is ≤0.8‰ for water and 0.9‰ when 200-pmol gas samples are analyzed, the latter improving to less than 0.1‰ with 4-nmol or larger gas samples.

A knowledge of the stable carbon isotopic ratio of methane δ^{13}C-CH_4 in natural systems can be useful in studies of the mechanisms and pathways of CH_4 cycling.[1] Unfortunately, because of the low CH_4 concentrations in many environments, analytical limitations have hindered such efforts. Methane samples have customarily been prepared for isotopic analysis off-line from the mass spectrometer used for analysis. In such systems CH_4 is oxidized in a glass combustion line and the resulting CO_2 is transferred cryogenically to an ampule for subsequent analysis,[2] which subjects the sample to the low efficiencies typical of mass spectrometer viscous-flow inlet systems.

Recent advances in isotope ratio monitoring gas chromatography/mass spectrometry (irm-GC/MS)[3] have allowed us to determine with greatly enhanced sensitivity the concentration and stable carbon isotopic ratio of CH_4 in natural systems. We previously reported on a system used to measure δ^{13}C-CH_4 in aqueous samples with moderate-to-high levels of CH_4.[4] We have subsequently modified this system for better performance, reliability, and reproducibility and developed a suite of new sample introduction systems that allows analysis at much lower concentrations and with a far wider range of sample types.

The sample introduction systems perform several functions. First, they match sample size with the limited range of analyte mass that the mass spectrometer can accept. Second, in the case of an aqueous sample, they extract the dissolved gases from the sample and insert them into a gas stream. Third, they remove water vapor and excess levels of CO_2 from the sample. Fourth, they keep the sample isolated from contaminants during injection. These new sample introduction systems, along with improvements to the basic analytical system, are the subject of the present paper.

EXPERIMENTAL SECTION

The basic analytical system, using an in-line NiO_2/Pt combustion reactor operated at 1150 °C, has been previously described[4] and is used with the newly developed sample introduction systems described below. However, we have found improved system reliability by heating the PLOT analytical column between runs. The column is operated at −20 °C in order to ensure baseline separation of the N_2, CH_4, and CO_2 peaks and is heated to 150 °C between runs to ensure that late-eluting peaks such as H_2O exit the column before the start of subsequent analyses. Commercial irm-GC/MS instruments generally require the use of a separate cryogenic column oven because the gas chromatographs included with such systems typically have the combustion furnace mounted through the wall of the chromatograph, thereby precluding subambient operation of the latter. Although a separate commercial gas chromatograph can be used as a column oven, we constructed a simple fan-circulated oven for this purpose. It is cooled using liquid CO_2 delivered from a pressurized gas tank and dispensed via a solenoid-operated valve (Varian Associates, p/n 03-917184-00), and heated with a 120-VAC resistance heater. Heating and cooling circuits are controlled by an Omega (Stamford, CT) Model CH8500 two-channel digital temperature controller.

Low-Level Water Samples (1−50 nM). We previously reported a method of analyzing water samples with CH_4 concentrations of >50 nM.[4] This detection limit is, unfortunately, too high for analyzing open-ocean seawater samples. The major limitations of the system are the inefficiency of the needle-based sparging procedure when used with volumes larger than a few tens of milliliters, water vapor transfer to the cryofocusing device when only a Nafion dryer is used, and sample contamination from small leaks in the system during long periods of gas sparging. We have therefore developed a sample introduction system for low-level aqueous samples (Figure 1). Analysis of these samples requires steps to minimize contamination and to maximize signal size. Thus, samples are collected in 125−300-mL bottles, and the entire contents of the bottles are directly delivered (via a minimum of connections) to a sparging column at the start of analysis.

The sparging column is constructed from a 3.5-cm-o.d. glass sealing tube with a coarse frit (Corning 39570-30C) and is used to extract the dissolved gases in the sample into the He stream, which is kept at a flow rate of ∼25 mL/min. The latter transfers the gases through a pair of polypropylene columns: the first, 25 cm × 6.4 mm o.d., packed with Drierite (10−20 mesh; Hammond

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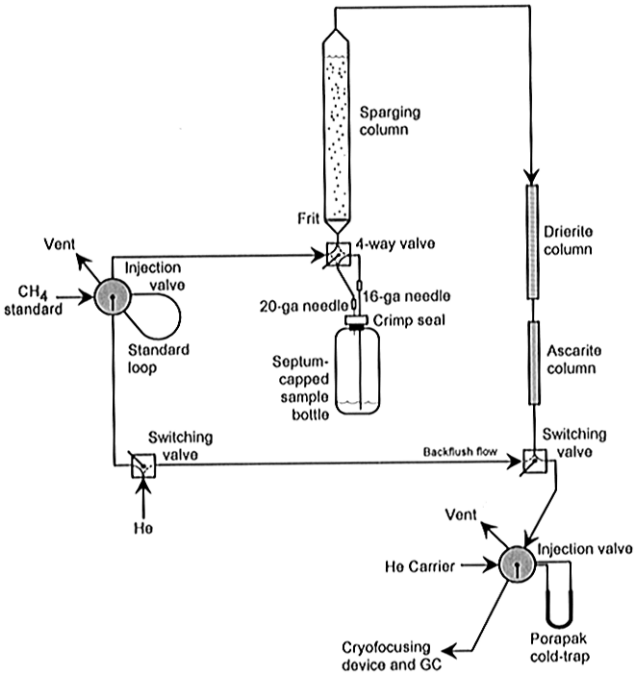

Figure 1. Schematic diagram of the sample introduction system used for low-level water.

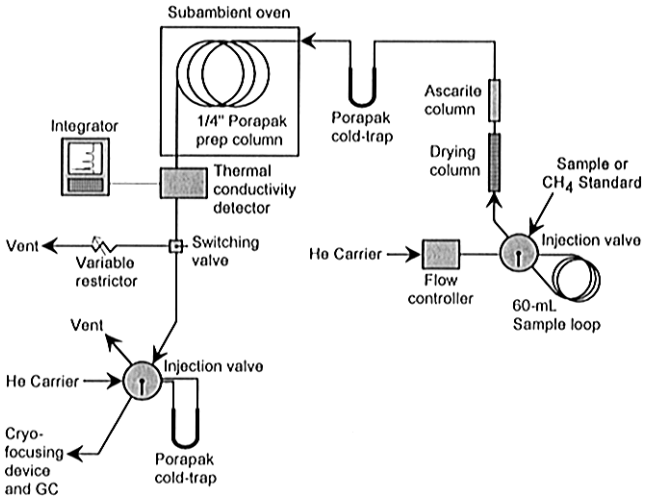

Figure 2. Schematic diagram of the sample introduction system used for low-level gas samples.

Drierite Co., Xenia, OH), and the second, 5 cm × 6.4 mm o.d., packed with Ascarite (20−30 mesh; Thomas Scientific, Swedesboro, NJ). The gases then pass through a stainless steel 25-cm × 3.2-mm-o.d. cold trap packed with Porapak-Q (80−100 mesh, Waters Assoc., Framingham, MA) located in the sample loop of the Hasteloy injection valve (Model C6UWEHC, Valco Instruments, Houston, TX). The four-way valve (Model 96779, Hamilton, Reno, NV) on the sparging column is used to divert the He stream through the sample bottle when the column is filled and, in conjunction with the backflush valves, to discard the sample after sparging.

Samples are processed as follows. The Porapak cold trap is immersed in liquid nitrogen, and its injection valve is switched to the "load" position. Then, with the four-way valve in the "load" position (dashed lines in Figure 1), first the 20-gauge needle and then the 16-gauge needle is inserted through the septum of the sample bottle; this causes the sample to be transferred to the sparging column. After the transfer is complete, the valve is switched to the "run" position (solid lines in Figure 1), and sample sparging begins. When sparging is completed (8 min for 200-mL samples), the cryofocusing segment upstream of the PLOT column is placed in liquid nitrogen, the Porapak injection valve is switched to the "inject" position, and the cold trap is placed in a tight-fitting aluminum heating block held at 100 °C (boiling water was used in ref 4). The sample gases are desorbed from the cold trap by the carrier and transferred to the cryofocusing segment; after 12 min the cryofocusing segment is moved to the adjacent 200 °C tube furnace, where the sample gases are desorbed and carried through the PoraPLOT analytical column for analysis. Note that an additional injection valve is located upstream of the sparging column to allow a CH_4 standard to be introduced into the sample sparging system after a sample has been processed. The sample is removed from the sparging column by removing the sample bottle and placing the 16-gauge needle into a waste receptacle, switching the four-way valve back to the "load" position, and temporarily turning the two switching valves to the "backflush" position (dashed lines in Figure 1) so that the sample is flushed out of the sparging column into the waste receptacle; the sample water can then be weighed to determine the sample size.

We generally collect and transport samples in glass serum bottles of up to 230-mL volume (Kimble) with grey butyl rubber septa (Pierce 12447) secured by aluminum crimp seals. When expecting long-term storage before analysis (longer than a few months), we have also stored samples in glass bottles sealed with ground-glass stoppers coated with Apiezon-N grease. Samples are preserved with aqueous saturated $HgCl_2$ solution: 0.5 mL/230-mL sample for open-ocean seawater; note that the toxicity of $HgCl_2$ necessitates the use of gloves when being handled.

Low-Level Gas Samples (45 ppb−5 ppm). Our previously described method[4] for analyzing mixed-gas samples with CH_4 concentrations of >5 ppm injected samples directly into the He stream immediately upstream of the cryofocusing device at the head of the PLOT column. This technique can also be used at concentrations as low as ~2 ppm if the major component in the gas is He (i.e., there are only trace levels of N_2 and CO_2). However, larger volumes of gas, necessary for analyzing lower CH_4 concentrations in mixed-gas samples, require the use of preparative-scale gas chromatography to separate CH_4 from the other gases in the sample prior to the injection and cryofocusing steps. This additional step is required because without it the large (and consequently wide) CO_2 and N_2 peaks eluting from the PLOT column would overlap the CH_4 peak, thereby preventing quantification of the CH_4 isotopic ratio by the data system of the irm-GC/MS (see ref 4).

Figure 2 shows the preparative GC sample introduction system developed for use with gas samples with molar mixing ratios of 45 ppb−5 ppm; injection volumes of up to 60 mL have been used with this system. After a gas sample has been loaded in the sample loop, it is injected using a flow-controlled He stream. H_2O and CO_2 are removed by passing the sample through 8-cm × 6.4-mm-o.d. and 6-cm × 6.4-mm-o.d. polypropylene columns packed with Drierite (10−20 mesh) and Ascarite (20−30 mesh), respectively. The remaining gases are trapped on a 25-cm × 3.2-mm-o.d. stainless steel column packed with Porapak-Q (80−100 mesh) at liquid N_2 temperature; this cold trap is fitted directly in-line with the He stream without the use of an isolation valve. Rapid heating of the Porapak-Q column to 100 °C desorbs the gases, which then pass through a 2.4-m × 6.4-mm-o.d. column packed

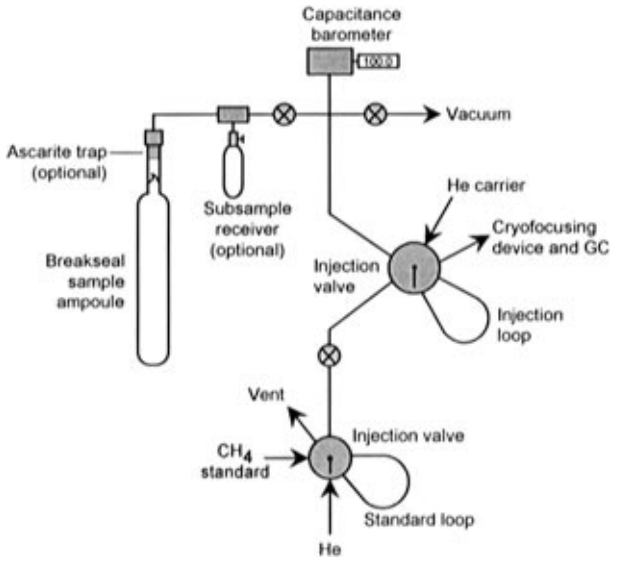

Figure 3. Schematic diagram of the vacuum manifold sample introduction system used for analyzing gas samples in sealed ampules.

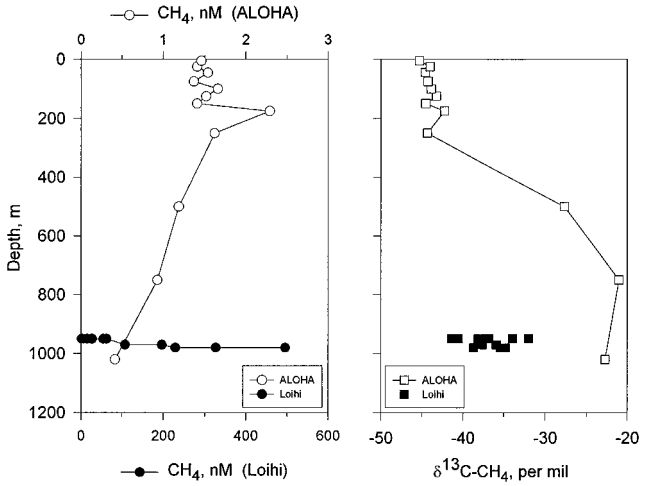

Figure 4. Methane concentration and isotopic ratio vs water depth in Loihi Seamount hydrothermal plumes and in background seawater collected at Station ALOHA during July 1995.

with Porapak-Q (80−100 mesh) held at subambient temperature (0 to −6 °C). This column allows baseline separation of N_2/O_2 from CH_4 in <4.5 min at a carrier flow rate of 60 mL/min; the separation is monitored using an in-line thermal conductivity detector and an integrator. The eluted CH_4 peak is trapped by diverting it to a 25-cm × 3.2-mm-o.d. stainless steel column packed with Porapak-Q (80−100 mesh) held at liquid N_2 temperature; this trap is located on the sample injection valve, as is the case for low-level water analyses. The cold-trapped CH_4 is then cryofocused and analyzed as described above.

Merritt et al.[5] previously described a preparative GC system for irm-GC/MS CH_4 analyses, but it requires 5-mL samples, which restricts the range of samples that can be analyzed. In addition, the preparative column is run at cryogenic temperatures (−118 °C) and the sample cryofocusing is performed on a 1-m length of capillary tubing.

Gas Samples in Rigid Containers. Samples of gas contained in sealed glass ampules or other rigid containers can be transferred to the sample injection loop by means of a low dead-volume vacuum manifold (Figure 3). The manifold can be used with samples that are either above or below atmospheric pressure, and allows repetitive analyses of a single sample if the mass of the sample is large enough (the technique we use requires a minimum of 200 pmol of CH_4/sample). The manifold is equipped with a capacitance barometer (Baratron 122A-1229, MKS Instruments, Andover, MA), thus allowing both the pressure and the volume of the gas in the sample loop to be determined immediately before injection. This technique has been used for analyzing CH_4 in the gases that had been vacuum-extracted from submarine hydrothermal vent fluids and transferred to glass break-seal ampules (Sansone et al., manuscript in preparation). It is also appropriate for the analysis of gas samples collected in evacuated rigid containers, which require some method of transferring the sample from the container to a sample loop.

The manifold is constructed from 1.6-mm-o.d. stainless steel tubing and stainless steel fittings with small internal diameters to minimize dead volume. Stainless steel inserts were also fabricated for the inlet tube on the manometer and the Cajon fittings (Crawford Fitting Co., Solon, OH) attached to the sample ampule and the barometer, again to reduce dead volume. When samples with elevated CO_2 levels are analyzed (such as hydrothermal vent fluid extracts), a 1-cm × 6.4-mm-o.d. polypropylene tube packed with Ascarite (20−30 mesh) is inserted in the outermost neck of the ampule before the latter is attached to the manifold.

Analysis begins by attaching a sealed sample ampule to the manifold and then evacuating the manifold to a constant vacuum with the injection valve in the "load" position and the He valve closed. The vacuum isolation valve is then closed, the vacuum level is monitored for the presence of leaks, and, if no leaks are detected, the break-neck seal on the ampule is broken with a magnet previously placed in the outer neck of the ampule. After the pressure in the system stabilizes, the ampule valve is closed, manifold pressure is recorded, and the sample is injected. The manifold can be re-evacuated and then refilled with sample if replicate analyses are desired; otherwise the manifold is repressurized with He and the used ampule removed. The number of replicate analyses possible is determined by the requirement to inject at least 200 pmol of CH_4/sample. As shown in Figure 3, a sidearm with a ampule (stopcock-equipped, if desired) can also be used to collect a split of the sample for additional analyses; we have used such samples for off-line preparation of CO_2 for δ^{13}C-CO_2 measurements. It should also be possible to use the vacuum manifold in Figure 3 to introduce samples into the preparative GC system shown in Figure 2. Such a system would allow the use of large sample loops needed for analyses of gases with very low concentrations of CH_4.

RESULTS

The methods described above have enabled several types of measurements to be made for the first time, primarily by allowing the analysis of much smaller sample volumes than have been possible in the past. Two examples of such applications are presented below.

Background Seawater and Seawater Hydrothermal Plumes

Figure 4 shows CH_4 and δ^{13}C-CH_4 data for seawater samples collected above a hydrothermal vent field located at a water depth

(5) Merritt, D. A.; Hayes, J. W.; Des Marais, D. J. *J. Geophys. Res.* **1995**, *100*, 1317−1326.

of 980 m on Loihi Seamount, HI;[6] these plume samples were collected with 5-L Niskin bottles during multiple submersible dives at distances of 10−30 m above different vents at the Pele's Vents field. Data from a "background" hydrocast taken ∼500 km away at Station ALOHA[7] are also shown for comparison.

The Station ALOHA data show that the surface mixed layer (∼200 m deep during this sampling) is slightly undersaturated in CH_4 with respect to the atmosphere. The seawater concentration is ∼1.5 nM, which is less than the value of 1.9 nM that can be calculated[8] for seawater in equilibrium with air with a CH_4 mixing ratio of 1.7 ppm (data from ref 9) at the insitu conditions (T = 26 °C; salinity 34.9). The δ^{13}C-CH_4 value of the mixed layer seawater is also offset from that of the atmosphere: the former is −44 to −45‰ (all isotopic ratios in this paper are vs PDB), whereas the latter is ∼−47‰ (e.g., ref 10). Although isotopic fractionation factors have not been determined for CH_4, one would expect dissolved CH_4 in air-equilibrated water to be enriched in ^{13}C relative to atmospheric CH_4. The offset is also consistent with the effects of isotopic fractionation associated with oxidation of CH_4 (e.g., ref 11) in the water column. The much more positive δ^{13}C-CH_4 values observed at greater depth are likely due to more extensive CH_4 oxidation during the aging of water masses below the mixed layer.

Figure 4 also demonstrates that δ^{13}C-CH_4 values can be useful for identifying hydrothermal inputs to water masses, as the stable isotopic signature of hydrothermal CH_4 (e.g., ref 12) can be significantly different from that of the local ambient seawater. The Loihi hydrothermal plume samples collected closest to the hydrothermal vents (i.e., the deepest samples shown) contained the highest CH_4 concentrations and had isotopic ratios of ∼−37‰. This value is similar to the −36.3‰ value measured for Loihi vent fluids collected at several vents in this area (Sansone et al., manuscript in preparation). Samples collected at points higher above the vents (i.e., at shallower depths) have lower concentrations and isotopic ratios that are both more and less negative than the vent fluids. The less negative values likely reflect a combination of (1) mixing of vent fluids with ambient seawater with more positive δ^{13}C-CH_4 values at these depths and (2) isotopic fractionation associated with aerobic CH_4 oxidation within the plume. The samples with more negative values are harder to interpret but may reflect previously undetected variability in the isotopic ratio of the vent fluids rather than processes occurring in the water column.

Boreal Forest Soils. We have used the low-level gas technique to analyze subatmospheric concentrations of CH_4 and associated δ^{13}C-CH_4 values in boreal forest soils (Figure 5). These samples were collected at the Bonanza Creek, AK, LTER site[13] by pumping gas from the loess soils using permanent probes deployed at various depths in the soil. The samples were stored in 6-L stainless steel flasks with a final pressure of 28 psi.

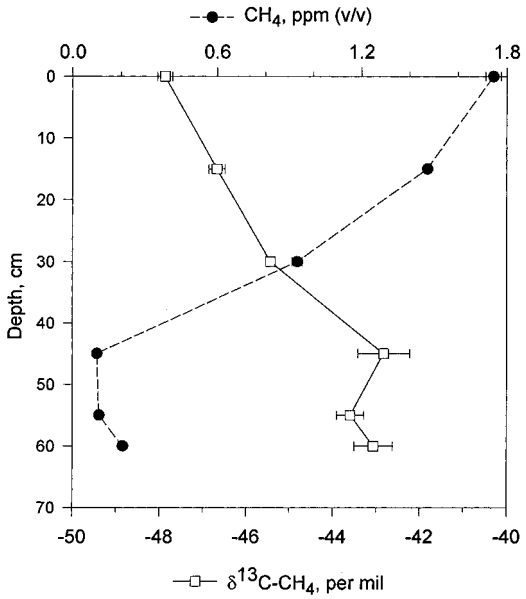

Figure 5. Soil gas CH_4 concentration and isotopic ratio vs depth in the upper, methane-oxidizing portions of boreal forest soils at a single site at Bonanza Creek, AK. The data are means and ranges of replicate analyses (n = 2−4) of individual samples; in some cases the horizontal range bars are smaller than the data symbols. Data shown at a depth of 0 cm are for an atmospheric sample collected 1 m above the ground. Samples courtesy of Dr. William Reeburgh, University of California—Irvine.

Previous work[14] showed that there is no CH_4 production in these soils and that there is consumption of atmospheric CH_4 down to levels of 0.1−0.02 ppm, with a maximum in CH_4 oxidation rate between depths of 10 and 20 cm. Our data (Figure 5) show that CH_4 oxidation actively occurs in the upper 40 cm of the soil, as reflected by CH_4 concentrations well below the atmospheric level of ∼1.7 ppm (data from ref 9). This loss of CH_4 is accompanied by a distinct shift to more positive isotopic values for the residual CH_4, which is consistent with the effects of biological oxidation (e.g., ref 11). Even at sub-ppm CH_4 concentrations (below a depth of 40 cm), the precisions of the concentration and isotope measurements (1 SD = 0.01 ppm and 0.6‰, respectively) are sufficient to discern the spatial characteristics of the profiles.

Precision and Accuracy. The precision of the seawater method was determined by analyzing replicate samples of open-ocean (1.5 nM) and coastal (25 nM) seawater. In the former case, the measured δ^{13}C-CH_4 value was −45.09 ± 0.76‰ (n = 7); in the latter case it was −60.70 ± 0.80‰ (n = 6). The precision of the low-level gas method was determined to be ±0.3‰ by analyzing 2.5−5 nmol of CH_4 from a tank of laboratory gas standard containing 99.2 ppm CH_4 in He (data not shown). The size and isotopic composition of the analytical blank was determined using the techniques of Gelwicks and Hayes.[15] In the case of the Bonanza Creek samples (Figure 5), the analytical blank ranged from 2 to 30% of the total concentration (sample + blank) of the samples analyzed. The δ-value of the blank (−44.1‰) was close to that of the samples, which resulted in a small correction to the isotopic composition of the samples (up to 0.6‰, but typically less than 0.1‰).

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Replicate seawater hydrothermal plume samples collected from the Endeavour Ridge[16] were analyzed (1) immediately after collection by shipboard GC with flame ionization detection using a headspace equilibration technique[17] (analyses courtesy of Dr. Marvin Lilley, University of Washington) and (2) after 8−9 months with the shore-based irm-GC/MS technique described here using samples stored in 240-mL bottles with ground-glass stoppers (samples courtesy of Dr. James Cowen, University of Hawaii). The results were well-correlated ($r^2 = 0.986$, $\text{conc}_{GC} = 1.07\ \text{conc}_{\text{irm-GC/MS}} - 0.046$, data not shown), indicating that sample storage did not introduce a systematic offset to the data and that our methods give results comparable to standard techniques in widespread use. Similarly, subsamples of gas from boreal forest soils at Bonanza Creek, AK, were collected in syringes and analyzed by GC in the field for CH_4 concentration and compared with analyses of replicate samples conducted 11 months later by irm-GC/MS. Again, the results of the two methods were well-correlated ($r^2 = 0.998$, $\text{conc}_{GC} = 1.04\ \text{conc}_{\text{irm-GC/MS}} - 0.085$, data not shown).

DISCUSSION

When combined with techniques we developed previously for higher concentration samples,[4] there are now on-line techniques available to examine the entire range of natural CH_4 concentrations in aqueous and gaseous samples. On-line methods offer several advantages over previous off-line methods, the most important being the much lower sample volumes required. For example, traditional off-line preparative techniques (e.g., refs 2, 18, and 19) would require ∼20 L of open-ocean seawater for a $\delta^{13}C\text{-}CH_4$ analysis instead of the 230 mL the method described here requires. This makes it feasible to conduct basinwide investigations of $\delta^{13}C\text{-}CH_4$ because it is now practical to collect large numbers of samples which can then be shipped to a shore-based laboratory for analysis. The maximum length of time that samples can be held prior to analysis is not currently known (experiments are presently underway in our laboratory); however, our experience with nonisotopic CH_4 analyses of open-ocean seawater indicates that storage periods of at least 2 years in ground-glass-sealed bottles do not result in measurable changes in CH_4 concentration (data not shown). The methods presented here are also faster than off-line techniques because samples are not transferred between preparative and analytical systems and because the cleanup and oxidation steps are quicker. In addition, chromatographic separation of the CH_4 being analyzed is an effective means of reducing chemical interferences. Finally, online methods allow the operator to detect the presence of interfering species by examining the mass chromatogram for a misshapen CH_4 peak; the latter would result from the coelution of an interfering compound with the CH_4 being quantified.

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