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Membrane Inlet Mass Spectrometer for Rapid High-Precision Determination of N_2, O_2, and Ar in Environmental Water Samples

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A membrane inlet mass spectrometer was modified to perform rapid, high-precision measurements of dissolved N_2, O_2, and Ar in water. The instrument pumps water at <1 mL min^{-1} through semipermeable microbore silicone tubing positioned inside an inlet vacuum line of a quadrupole mass spectrometer. Precise pumping and temperature control of the water sample contribute to high signal stability and reproducibility. Dissolved gas concentrations are determined from intensities of the mass spectrometer signals in the multiple ion detection mode. Precision (coefficient of variation) is <0.5% for N_2, O_2, and Ar concentrations and <0.05% for N_2/Ar and O_2/Ar ratio data. Deviation from expected values was between 0.5 and 1.5% for air-equilibrated water of widely ranging temperature and salinity. Advantages of the instrument over existing methods include rapid throughput (~20–30 samples h^{-1}), lack of sample water preparation (e.g., no degassing step), small sample size (<10 mL), and high-precision measurement of both concentration and gas ratio data. A limitation of the present instrument is the difficulty in measuring water with significantly supersaturated gas concentrations. An example of the utility of the instrument is described for the measurement of denitrification in estuarine sediment.

Dissolved gases play an important role in marine and aquatic science. Deviations from equilibrium concentrations are common and reflect biological activity and physical processes acting on the system. Of the three most concentrated gases in aerobic waters, N_2 and O_2 are affected by both biological and physical processes, whereas Ar is affected strictly by physical processes. This distinction has enabled limnologists and oceanographers to separate physically driven processes, such as bubble release or injection, from biological processes, such as net biological oxygen exchange.[1-3] In some cases, only small fractional (<1%) deviations from equilibrium occur and it is necessary to measure dissolved gases with high precision. For that, mass spectrometers, which provide highly precise gas ratio data (e.g., N_2/Ar ratios), are usually used.

The principal methods of analysis of the major dissolved gases include gas chromatography (GC) or mass spectrometry (MS). GC provides direct concentration data while MS provides gas ratio data, which can be converted to concentration data with the independent measurement of concentration of one of the component gases (e.g., O_2). Gas chromatography offers a precision of measurement in the range of 0.3–1% for N_2, O_2, and Ar extracted from water.[1] Mass spectrometry, on the other hand, yields precision on the order of 0.05% for gas ratios (e.g., N_2/Ar or O_2/Ar).[3] Both GC and MS require injection of a gas sample that was previously extracted from the water sample in a separate batch process. This separate extraction step adds time and can limit overall precision of the gas analysis.[3]

The instrument described here is a type of membrane inlet mass spectrometer (MIMS)[4,5] optimized for high-precision measurement of the dominant dissolved gases in water. It uses a capillary bore silicone tube interface in a configuration modified from a design reported previously by Bier and Cooks.[6] We considered desirable characteristics of the instrument to include high-precision measurement of dissolved N_2, O_2, and Ar concentrations and selected gas ratios (e.g., N_2/Ar), rapid end-to-end sample analysis, and small sample volume requirement. These were met by incorporating components that provided temperature and flow control of the water sample and trapping of interfering gases. Compared to other methods of measuring the dominant dissolved gases in water, the MIMS instrument is unique in the fact that it analyzes a water sample directly, thus avoiding a separate time-consuming degassing step. Degassing the water sample is done via the membrane interface, and gases are analyzed in-line by the mass spectrometer. Response time of the analyzer is rapid and throughput of 20–30 samples h^{-1} is possible. Moreover, precision of estimates of N_2, O_2, and Ar concentrations and gas ratios compare favorably with those obtained with existing optimized GC and MS methods.

MATERIALS AND METHODS

Instrumentation. A membrane inlet mass spectrometer was modified from existing types by including a highly stable flow of water across the membrane, temperature control of the water in the membrane, and cryogenic trapping of water vapor and other compounds (e.g., CO_2) that may interfere with the detection of the gases of interest. Our evaluation considered measurement of N_2, O_2, and Ar, only, although this technique may also be appropriate for measurement of other dissolved gases.

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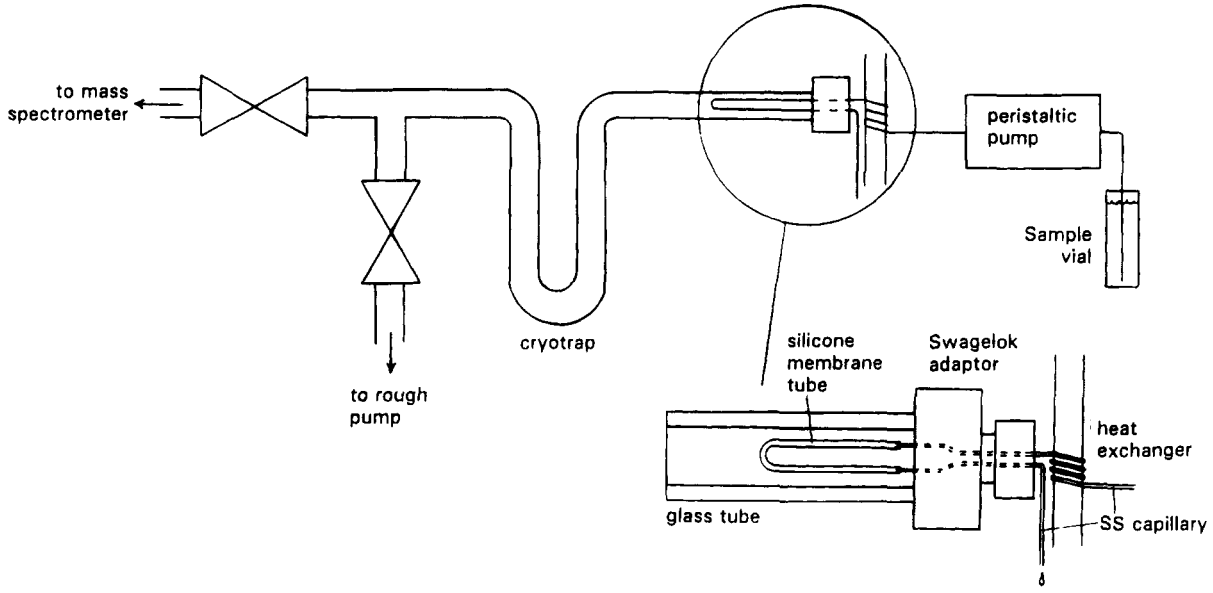

Figure 1. Schematic of the sample pumping system and vacuum interface to the quadrupole mass spectrometer.

The instrument consists of the following functional systems: water pumping, vacuum inlet, MS detector, and computer controller. The water pumping system (Figure 1) uses a peristaltic pump (Rainin Rabbit) operating at a constant speed (~0.75 mL/min). The water line upstream of the membrane interface consists of stainless steel (SS) capillary tubing connected to Viton pump tubing. Viton pump tubing provides a more rapid equilibration of the system than PVC tubing because of its lower gas permeability and reduced carryover of gases between samples. The stainless steel capillary that leads from the pump to the membrane inlet is wrapped and cemented around a 6.4 mm diameter copper tube through which thermostated water flows. This serves as a heat exchanger which stabilizes sample temperature to within 0.1 °C. The selected temperature is arbitrary, though it is usually set to a value close to 20 °C. After the heat exchanger, the capillary immediately extends through a vacuum lock (Swagelok fitting) and is connected to a 5 cm long section of 0.3 mm i.d. (0.15 mm wall thickness) silicon tubing (Silastic, Dupont). The silicone tubing serves as a semipermeable membrane interface. Sample water exits the vacuum lock through a second stainless steel capillary.

The vacuum inlet system (Figure 1) is built around a section of 9 mm diameter glass tubing bent to form a U-tube with horizontal exits. Gases that diffuse through the membrane located at one end of the tube pass through a liquid nitrogen trap (U-tube) to remove water vapor and CO_2 before entering the mass spectrometer. (CO_2 can affect N_2 analyses because of its CO (mass 28) fragment.)

Gases were detected using a quadrupole mass spectrometer (Balzers QMG420 with cross-beam ion source and secondary electron multiplier) operating in multiple ion detection mode. A pumping speed of 9 L s^{-1} was determined by a 1 cm orifice located above a 50 L s^{-1} turbomolecular pump. The ion source was tuned to provide relatively broad peaks with near-flat tops. Masses 28, 32, and 40 were detected sequentially in a repetitive cycle (~1.5 Hz) and signals were analyzed using customized software. Time trends of the mass signals and ratios of selected pairs are displayed as boxcar averages of 50 data points. Included is a visual display of each mass signal and calculated ratio magnified by ignoring the two most significant digits of each datum and scaling the remainders between 0 and 1. This provided an accurate visual assessment of the stability of the signal at a resolution of 10^4. Termination of an individual analysis was on operator command based on a visual assessment of signal stability. On termination of a sample analysis, the 50 point moving average of each signal and ratio was recorded. Sample changeover involved stopping the peristaltic pump for 2–4 s while vials were exchanged.

Sampling. Water samples were collected in 8 mL screw-capped vials using Teflon-faced silicone septa as closures. Vials were filled by siphon to overflowing, capped with no head space, and stored under water at room temperature until analysis. Samples remained uncontaminated by leakage through the septa for at least 24 h, but were generally analyzed within a few hours of collection. Samples were analyzed by inserting a stainless steel dip tube to the base of an uncapped vial, thus drawing off water from the base only. This extended the length of time the instrument could dwell on a sample before surface contact with air altered the gas composition of the water that was sampled. A high depth/diameter ratio of the sample vial is preferred. Other methods of sample withdrawal were investigated in an effort to increase the analysis time before contamination occurred, including the use of 125 mL conical flasks and injection of cold brine as a displacement solution in closed septa vials. Those changes were advantageous in situations where sample-to-sample gas compositions differed greatly and analysis time needed to be extended to several minutes. For all analyses reported here, samples were withdrawn using the simple open vial method.

Standards. Standards consisted of air-equilibrated water maintained at constant temperature and salinity for >72 h. Salinity gradients were made with either NaCl in deionized water or diluted ocean water. Water was maintained in either temperature-controlled environment rooms or jacketed flasks regulated by thermostated water. Flasks were covered with plastic wrap or loosely stoppered to maintain 100% relative humidity and limited air exchange. Dissolved gas concentrations were determined from equations derived by Weiss.[7] Variable O_2 standards were prepared

(7) Weiss, R. F. *Deep-Sea Res.* **1970**, *17*, 721–735.

by adding different amounts of sodium dithionite to water standards. Vials were immediately capped, mixed, and analyzed by the standard protocol.

Sediment Core Denitrification Experiment. The instrument was used to measure metabolic processes within a brackish water sediment system. Sediment samples were collected in triplicate from an experimental pond on the campus of Horn Point Environmental Laboratory using a 15 cm diameter cylindrical plexiglass coring device. Approximately 10–15 cm deep cores with overlying water (~2 L) were transferred to a filled aquarium inside a temperature-controlled (20 °C) environment room. Each core was fitted with inlet and outlet tubes to provide continuously flowing water at 3 mL m^{-1}, representing a turnover time of ~11 h inside each core. Feed water was collected from the ponds at least 24 h before use, filtered using 5 μm mesh, and stored in the environment room with gentle bubbling. The head-space water was stirred continuously (60 rpm) using a magnetic bar.

Duplicate water samples (8 mL) were collected from inlets and outlets at daily intervals and analyzed immediately for N_2, O_2, and Ar concentrations and N_2/Ar and O_2/Ar ratios. Data were analyzed by using differences in N_2/Ar ratios between inlet and outlet water. Ar concentration was assumed to be at equilibrium with the water at the measured temperature and salinity. N_2 flux (F) was calculated as $F = (C_i - C_o)V/A$, where C_i and C_o are the concentrations (μmol/L) of N_2 in the inlet and outlet, respectively, V is the flow rate (L/h), and A is the sediment surface area (m^2).

RESULTS AND DISCUSSION

The original design criteria—high-precision, high-throughput, and small sample size—were met with the instrument as described here. Several components contributed to the high precision including, a stable liquid pump, sample temperature control, and cryotrapping. The peristaltic pump operated in a range where the signal intensity depended on pump speed. Short-term stability was high, but drift in the signal intensity on the order of a few percent or less per hour occurred due to "aging" of the pump tubing. This invariably caused lower signals over time (ratio data were generally unaffected, however). Drift in the concentration data was accounted for by periodically analyzing standards and correcting for changes in instrument sensitivity during the course of an analysis run. The time required for mass signals to stabilize depended on flow rate. In general, increased flow rate reduced the time to steady state. But increasing flow also decreased the time that a vial sample could be analyzed before air-contaminated water reached the dip tube. A flow of ~0.75 mL m^{-1} was a suitable compromise, resulting in sample turnaround as brief as 90 s.

Precision and accuracy were evaluated on temperature and salinity standards. The practical lower limit of a temperature standard was ~10 °C when the instrument was operated at room temperature (~24 °C). This was due to the negative pressure developed in the capillary tube leading to the pump and the resultant outgassing of the supersaturated water in the feed line. Results of five temperature standards using full strength seawater are presented in Table 1. For measurement of concentration, coefficients of variation (CV) averaged 0.20% for five temperatures and three gases. Coefficients of variation of N_2/Ar and O_2/Ar ratios averaged 0.068%. Accuracy was assessed by standardizing the instrument at a single temperature (20.9 °C). Concentration values were generally within 1.5% of expected, and ratios were within 0.5% of expected. In a similar exercise, variable salinity water of constant temperature was analyzed (Table 2). Measurement precision (CV) was comparable to variable temperature standards, averaging 0.20% for the three gases and six salinities and 0.030% for the N_2/Ar and O_2/Ar ratio measurements.

Table 1. Dissolved Gas Analysis of Seawater (36 ppt Salinity)[a]

	temp, °C				
	12.7	17.6	20.9	30.6	39.3
N_2					
mean (μM)	473.0	432.7	415.7	359.3	325.6
CV (%)	0.05	0.13	0.44	0.07	0.25
expected (μM)	479.7	439.3	415.7	359.3	320.3
Δ (%)	−1.41	−1.49	0	0.01	1.65
O_2					
mean (μM)	260.5	236.4	225.6	190.5	169.3
CV (%)	0.08	0.15	0.39	0.10	0.48
expected (μM)	264.6	239.9	225.6	191.5	168.3
Δ (%)	−1.58	−1.47	0	−0.51	0.62
Ar					
mean (μM)	12.82	11.60	11.05	9.35	8.31
CV (%)	0.05	0.10	0.37	0.10	0.21
expected (μM)	12.94	11.75	11.05	9.40	8.26
Δ (%)	−0.94	−1.27	0	−0.46	0.66
N_2/Ar					
mean (μM)	36.89	37.30	37.61	38.41	39.16
CV (%)	0.03	0.02	0.08	0.02	0.08
expected (μM)	37.08	37.39	37.61	38.23	38.79
Δ (%)	−0.52	−0.24	0	0.45	0.96
O_2/Ar					
mean (μM)	20.32	20.38	20.41	20.37	20.36
CV (%)	0.05	0.06	0.03	0.00	0.31
expected (μM)	20.45	20.42	20.41	20.38	20.38
Δ (%)	−0.66	−0.23	0	−0.07	−0.07

[a] The analyzer was calibrated using 20.9 °C water. Quadruplicate samples were analyzed in the following order: 20.9, 39.3, 30.6, 17.6, and 12.7 °C. Run time for completion of the 20 samples was 32 min. The percent deviation (Δ) between the mean value and expected value was calculated as (mean − expected)/expected × 100.

The influence of different O_2 concentrations on the N_2 signal and N_2/Ar ratio was investigated by altering the amount of O_2 in water standards equilibrated at constant temperature (Figure 2). Over the range of 0–100% O_2 saturation, the N_2 signal increased proportionally 2.5%, while the N_2/Ar ratio remained constant.

The application that we present deals with the measurement of denitrification, a microbial process that reduces NO_3^- to N_2 and that is often associated with anaerobic marine sediments. Developing methods that directly measure the end product has been hampered by the low flux of N_2 compared to its background concentration. This requires routine precision at better than 1%. Hence, alternative methods have been employed which either require inhibitors or other manipulations that modify the system and/or place it in a non-steady-state mode which variably cause alterations in the in situ denitrification flux.[8] By exploited the high-precision measurement capability of the instrument, we could utilize an experimental design that offered the advantage of manipulating the system among various steady-state conditions.

Figure 3 illustrates the result of a continuous-flow experiment on triplicate brackish pond sediment cores with overlying water. Net N_2 evolution (net denitrification) ranged between 1 and 60 μmol m^{-2} h^{-1} depending on the addition of nitrate or nitrate plus acetate (an energy source). These fluxes are in the range of denitrification rates found in estuarine sediments measured by other techniques.[9] In our experiment, flow rate was adjusted to

(8) Seitzinger, S. P.; Nielsen, L. P.; Caffrey, J.; Christensen, P. B. *Biogeochemistry* **1993**, *23*, 147–167.

Table 2. Dissolved Gas Analysis of Water of Differing Salinities (20 °C)[a]

	salinity, ppt					
	0	2.25	4.5	9.0	18.0	36.0
N_2						
mean (μM)	524.2	523.8	516.4	503.9	480.6	439.5
CV (%)	0.22	0.04	0.21	0.15	0.30	0.16
expected (μM)	531.2	523.6	516.1	501.4	473.4	421.9
Δ (%)	−1.31	0.04	0.06	0.49	1.53	4.19
O_2						
mean (μM)	279.7	279.9	276.4	270.1	258.1	237.6
CV (%)	0.24	0.04	0.18	0.13	0.24	0.12
expected (μM)	283.6	279.9	276.2	269.0	255.1	229.3
Δ (%)	−1.39	−0.00	0.06	0.42	1.21	3.62
Ar						
mean (μM)	13.70	13.71	13.53	13.23	12.67	11.69
CV (%)	0.22	0.03	0.20	0.12	0.26	0.14
expected (μM)	13.91	13.73	13.54	13.18	12.50	11.24
Δ (%)	−1.49	−0.17	−0.09	0.30	1.39	4.08
N_2/Ar						
mean (μM)	38.21	38.16	38.13	38.05	37.89	37.57
CV (%)	0.003	0.014	0.027	0.034	0.039	0.020
expected (μM)	38.19	38.15	38.11	38.03	37.87	37.55
Δ (%)	0.06	0.04	0.06	0.05	0.07	0.06
O_2/Ar						
mean (μM)	20.39	20.40	20.41	20.39	20.35	20.31
CV (%)	0.052	0.006	0.027	0.014	0.040	0.033
expected (μM)	20.39	20.39	20.40	20.40	20.40	20.41
Δ (%)	−0.02	0.00	0.06	−0.02	−0.24	−0.48

[a] Duplicate samples were analyzed in ascending order of salinity followed by the analysis of a second set of duplicate samples in random order. The analyzer was calibrated using 2.25 ppt water at the beginning and middle of the run. N_2/Ar and O_2/Ar data were corrected for analyzer background according to equation $(G - G_b)/((G/[G/A]) - A_b)$, with G the gas (N_2 or O_2) concentration signal, G_b the analyzer background signal, A the argon concentration signal, A_b the argon background signal, and $[G/A]$ the gas ratio value from the sample run. The percent deviation (Δ) between the mean value and expected value was calculated as (mean − expected)/expected × 100.

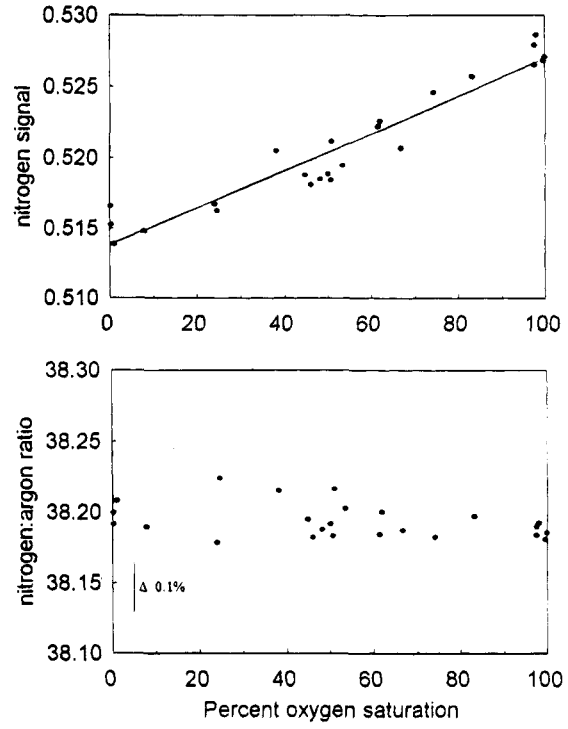

Figure 2. Effect of different oxygen concentrations on the measurement of N_2 (top panel) and N_2/Ar ratios (bottom panel). Line corresponds to the linear best fit.

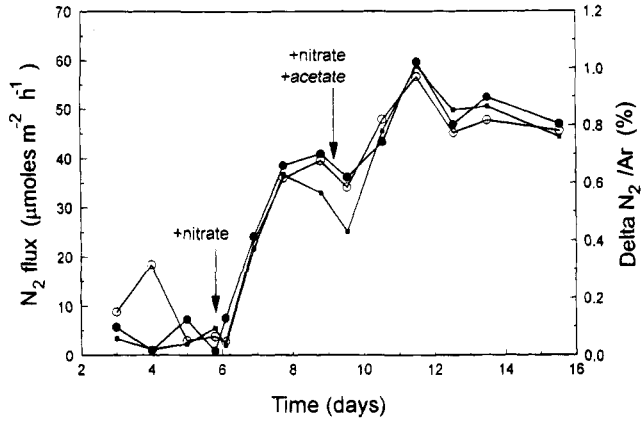

Figure 3. Time course of triplicate sediment cores exposed to continuous-flow conditions. See text for details.

maintain the O_2 concentration in the overlying water at ~50% of saturation (data not shown), which had the desirable effect of maintaining oxygenated headwater throughout the 16 day experiment. This parameter constrained the ΔN_2 to a value between 0 and 1% owing to the significantly lower denitrification flux compared to respiratory flux in the system. Therefore, resolution of the change in N_2 required use of N_2/Ar ratios that could be measured with higher precision than N_2 concentrations alone. This is the first demonstration of the use of direct N_2 production measurements in steady-state sediment cores.

We have identified both advantages and disadvantages of the MIMS instrument over conventional methods for measuring dissolved N_2, O_2, and Ar. The MIMS instrument offers significantly higher analysis speed with >100 analyses per day being readily attainable. This has advantages in environmental studies where spatial and temporal variability can be high and intensive sampling is desirable. Additionally, it can be exploited where rapid, near real-time data are beneficial, such as in kinetic experiments or where contingency-based decisions would improve efficiency of sampling, such as in shipboard oceanographic projects. (Our mass spectrometer has been operated aboard oceanographic research vessels, indicating the potential for using the instrument at sea.)

The MIMS instrument also provides data equivalent to two different instruments, a GC and an MS. Generally, for the highest precision work, dual-inlet isotope ratio mass spectrometers have been used, although the level of precision is ultimately limited by the step involving degassing of the water sample and not the analysis of the gas itself. Such mass spectrometers are not widely available owing to their expense and technical requirements. The quadrupole mass spectrometer used in the instrument is significantly less expensive to purchase and maintain and can be assembled as a benchtop instrument.

Limitations of the instrument include its difficulty in measuring supersaturated water and lack of absolute standards based on gravimetrically or volumetrically prepared standards. Bubbles originating from outgassing of supersaturated water cannot be tolerated in the silicone capillary tubing because they perturb the uniform gas flow across the membrane. The instrument operated at room temperature, which limited cool water standards to a temperature of >9 °C. Such samples could withstand warming and the negative pressure developed within the capillary dip tube without outgassing, whereas cooler water standards outgassed in the fluid inlet line. Supersaturated water is also found in aquatic

(9) Seitzinger, S. P. *Limnol Oceanogr.* **1988**, *33*, 702–724.

samples that have high oxygen production rates or bubble injection (e.g., under dams). Those samples can also be difficult to measure with the instrument although we have successfully measured 27 °C pond samples with 200% oxygen saturation. It should be possible to extend the capability of the instrument for measuring low-temperature samples (0−10 °C) by operating the peristaltic pump and inlet tubes at subambient temperature by use of a refrigeration box.

Calibration of the instrument depends on standards consisting of air-equilibrated water of known temperature and salinity. This type of standard will have an effect on the accuracy of measurement depending on how rigorously the standards are prepared. In general, one can expect the solubility values derived from published tables or regression equations to be accurate to within a few tenths percent for equilibrated water under standard conditions (100% relative humidity and 1 atm). Therefore, measurements by the MIMS instrument cannot exceed this level of accuracy. In the present experiments, atmospheric pressure was not measured or controlled over the head space of the standard water samples. This would affect estimates of concentration but not ratios, as changes in pressure would affect gases similarly. Yet, the lack of pressure measurement or control should not affect our determination of accuracy statistics since all standards from a given temperature or salinity gradient were sampled at the same time resulting in, at worst, a deviation from the true value by some constant value. Standards used in this study were all exposed to a similar atmospheric pressure history. Nevertheless, it is apparent that when high accuracy is required, pressure control or measurement during the preparation of standards is necessary.

We believe the instrument described here offers significant advantages over existing methods when high precision and/or rapid analysis is desired. We have demonstrated its ability to measure small differences in N_2 in environmental samples in a rapid and convenient way. The continuous-flow inlet, described here, is rudimentary and can be modified in various ways, for example, to provide automated sample handling or continuous monitoring of one or more experimental systems (e.g., tanks). Additionally, the quadrupole mass spectrometer can measure other dissolved gases (e.g., CO_2 and CH_4, among others) and isotopes (e.g., $^{18}O_2$ and $^{15}N_2$, among others) as previously demonstrated with existing membrane inlet mass spectrometers.

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