RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2002; **16**: 1257–1260
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/rcm.711

Rapid ^{18}O analysis of small water and CO_2 samples using a continuous-flow isotope ratio mass spectrometer

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Received 4 March 2002; Revised 30 April 2002; Accepted 1 May 2002

High-frequency throughput is often needed in isotopic studies in biological and medical fields. Here we report that high-precision oxygen isotope ratio measurements of water (±0.13‰) were rapidly and routinely made on small samples (40–100 μL) using an isotope ratio mass spectrometer operated in continuous-flow mode. Simple modifications to existing instrumentation allow for rapid manual analyses of dilute CO_2 (10% CO_2/90% N_2), including the addition of a septum port and water trap prior to the gas chromatography (GC) column (elemental analyzer column in this study) and the extension of fused-silica capillary tubing between the mass spectrometer source and the effluent tubing from the GC column (located within the CONFLO unit on Finnigan mass spectrometers). We routinely analyzed 20 small-volume samples per hour using this technique, without sacrificing precision of the oxygen isotope ratio measurement. Copyright © 2002 John Wiley & Sons, Ltd.

Evapotranspiration, atmospheric humidity, insect fluids, animal breath, leaf water, and soil pore water are just a few examples where numerous water samples are needed for analysis in biological research, yet the total amount of water available can be small. In many biological studies, sample sizes of ~60 μL water are typical and this creates a challenge for oxygen isotope ratio analyses using traditional methods which measure cryogenically isolated CO_2 from equilibrated CO_2/H_2O mixtures on the dual-inlet of mass spectrometers.[1] Sample sizes used for such a technique are normally 1–5 mL, which is acceptable for multiple analyses in geochemical and hydrological research where large reservoir sizes are available. The analysis-preparation time using traditional approaches can also be long because of the need to both equilibrate and then isolate the CO_2 gas used in the analysis. Yet the high precision afforded by this technique (±0.05‰) is often critical.[2,3] Recent advances in the equilibration apparatus and sample introduction into the dual-inlet of a mass spectrometer,[2,4] guanidine hydrochloride extraction,[5] and *in situ* ^{18}O equilibrations[6] have allowed for analyses of small-sized water samples (as low as 10 μL) with precisions of ±0.1‰. However, the time required for processing a single measurement can still be limiting for many biological studies, which often require large numbers of analyses. Here, we describe a method to rapidly process small water samples (as low as 40 μL) with high precision. Through modifications to existing hardware (CONFLO interface), these measurements can be made in laboratories using a continuous-flow isotope ratio mass spectrometer (CF-IRMS).

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EXPERIMENTAL

Instrumentation

This method was developed using an isotope ratio mass spectrometer equipped with an elemental analyzer (EA) (model 1108; Carla Erba, Milan, Italy) coupled to a dual-inlet isotope ratio mass spectrometer (Finnigan MAT Delta S; Bremen, Germany) through an open split (CONFLO II; Finnigan MAT, Bremen, Germany) (see Fig. 1). The source of the mass spectrometer is held at a pressure of 1×10^{-6} mbar (in continuous-flow mode) and an acceleration voltage of 3 kV. The elemental analyzer GC column is 6.4-mm o.d., 2-m long, packed with Porapak-Q, and held at 60 °C with a helium carrier gas flow of 80 mL/min. A 2-mm o.d. stainless steel tube carries the effluent from the EA gas chromatograph column to the CONFLO II interface open split, entering through one end of a 6-mm o.d. glass tube. Within this 6-mm glass tube, an internal 2-mm o.d. glass tube is housed where a second He source and various calibration gases can be introduced into the He carrier stream. The carrier stream then sweeps these gases (if used) plus the elemental analyzer effluent past a sipping fused-silica 0.32-mm o.d. capillary tube, which is attached directly to the source of the mass spectrometer (Fig. 1). As a result of the configuration of the CONFLO II interface open split, the mass spectrometer sipping capillary has access to only a small portion of the effluent from the EA.

Modifications of the CF-IRMS setup

Four modifications were made for on-line analysis of dilute

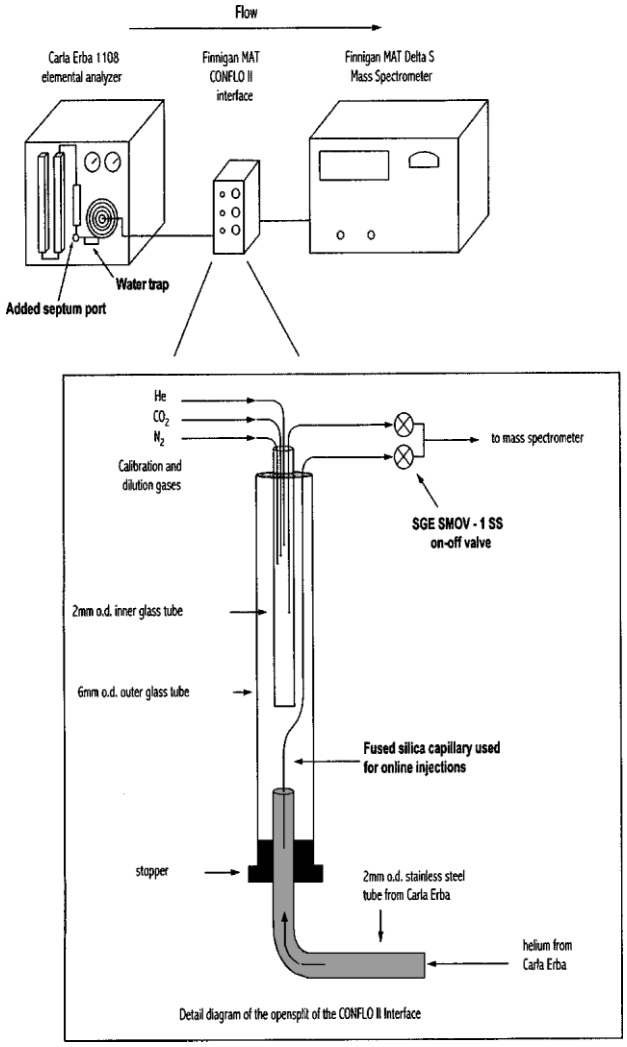

Figure 1. Schematic of the continuous-flow setup with an inlay of the open split within the CONFLO II interface. Modifications are circled.

CO_2: (1) a 6.4-mm stainless steel septum port equipped with a two-layer, silicone/Teflon septum (part # 79451; Alltech, Deerfield, IL, USA) was placed in-line, prior to the elemental analyzer GC column and after the reduction furnace; (2) a H_2O drying trap (6.4-mm o.d. by 10-cm long magnesium perchlorate trap held at room temperature) was installed immediately after the septum port; (3) a new fused-silica 0.32-mm o.d. capillary tube was placed into the He effluent from the elemental analyzer by inserting one end directly into the 2-mm o.d. stainless steel tube as it enters the open split; (4) an additional on/off valve (model SMOV-1 SS; SGE, Inc.) valve was placed in-line for ease of sample introduction into the mass spectrometer source. Modifications to the system setup are in bold type within Fig. 1.

Water sample preparation and analysis

A modified method for H_2O/CO_2 equilibration is used to perform $\delta^{18}O$ analyses on small samples.[7] Specifically, small volumes of water (0.1–0.5 mL) are allowed to equilibrate with dilute CO_2 (10% in a background of N_2) in 6-mL glass vials (part # 66126; Alltech, Deerfield, IL, USA) sealed with a 20-mm Hycar stopper (part # 6683; Alltech). The vials are first flushed with a CO_2/N_2 mixture (10%/90%), capped immediately with the Hycar stopper, and sealed with 20-mm aluminum seals (part # 98807; Alltech) allowing pressures to remain at atmospheric values inside the vial. Water is then introduced into the sealed volume with a sterile 1-mL insulin syringe (Becton, Dickinson & CO, Rutherford, NJ, USA), and the CO_2/H_2O mixture is allowed to equilibrate for 48 h[2,4] at room temperature.

After equilibration, the samples are analyzed using the CF-IRMS. A 1-mL gas-tight lockable syringe (model A2-VICI; Precision Sampling, Baton Rouge, LA, USA) is used to inject 400 μL of the CO_2/N_2 mixture from the headspace of the equilibration vials into the He stream of the elemental analyzer (through the new septum port). Samples are injected at an interval of 150 s and 12 samples are analyzed during a typical 30-min run. During the analysis, a calibration gas (pure CO_2 calibrated against V-SMOW) is first introduced through the dual-inlet bellows. Then ten unknowns and two laboratory standards (spanning a $\delta^{18}O$ range of 12.3‰ and encompass unknown values) are injected. Finally, a second introduction of calibration gas from the bellows is made. The oxygen isotope values for the unknowns are calculated by normalization to the laboratory standards injected during the same run.

This modification allows for $\delta^{18}O$ analyses to be made on equilibrated CO_2 taken directly from the headspace of an equilibration volume without the need for prior cryogenic CO_2 isolation. Also, this method allows for H_2O/CO_2 equilibration to take place on the laboratory bench and does not require a water bath, as long as standards and unknowns are subjected to identical conditions and room-temperature fluctuations are moderate (~1°C).

RESULTS AND DISCUSSION

System linearity

The $\delta^{18}O$ values of equilibrated CO_2 were insensitive to the amount of sample CO_2 injected into the inlet port above a threshold of 16 Vs. From one headspace vial, CO_2/N_2 gas volumes ranging from 10–600 μL were injected into the inlet port and analyzed on the mass spectrometer. These volumes

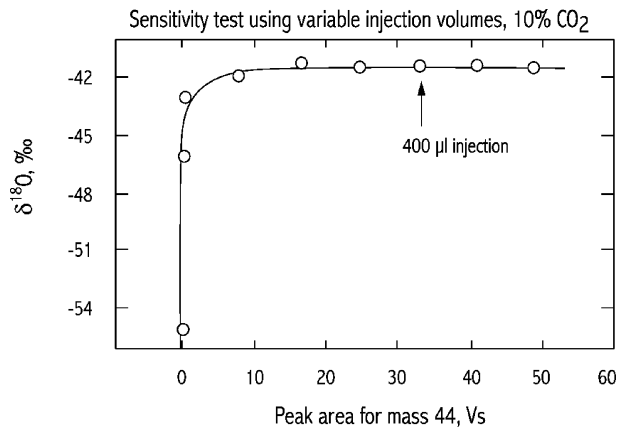

Figure 2. Sensitivity test using 50–600-μL injection volumes of 10% CO_2/90% N_2. Non-linear response of the machine is seen with small injection volumes (up until 200 μL).

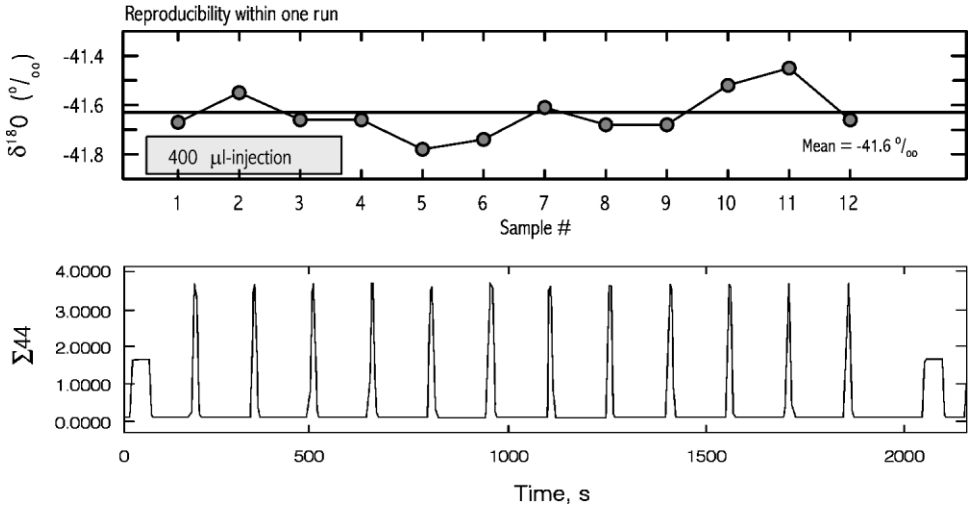

Figure 3. Analysis of the variation within $\delta^{18}O$ and the voltage response from the mass 44 peak. Repeat 400-µL injections of 10% CO_2/90% N_2 were made in one run with a precision of ±0.12‰ indicating analysis precision for dilute CO_2 samples.

corresponded to peak areas of 0.5–49 Vs and peak amplitudes of 0.06–5.4 V (for mass 44) on this mass spectrometer. Poor signal/noise ratios only occurred below 16 Vs associated with small sample sizes (<200 µL gas volume); the $\delta^{18}O$ value was then observed to decrease by as much as 1.42‰ below this threshold (Fig. 2). The Faraday cups became saturated at peak areas above 64 Vs (or 7 V for mass 44, data not shown), thereby defining a window of operation. These non-linear responses occur in most isotope ratio mass spectrometers and optimization of operation should be established, especially when small sample sizes are analyzed. For our typical analysis, we strove for a sample size giving a peak area of 30 Vs (400-µL injection volume).

Precision within a run

Repeat 400-µL injections of a 10% CO_2/N_2 mixture (housed in a 100-mL glass flask equipped with a two-layer silicone/Teflon liner) were conducted to examine the analysis precision within a single run (Fig. 3). In this study, the $\delta^{18}O$ of the gas mixture averaged −41.62‰ and the analysis precision was ± 0.12‰.

Accuracy and precision across runs

To determine the precision and accuracy of the method, an analysis was conducted on repeated samples from different runs. Six water samples were used: (1) Salt Lake City UT ground water (with known value of −15.50‰ relative to V-

Table 1. Test of precision of $\delta^{18}O$ in various water samples by on-line analyses

	On-line GC-IRMS Analysis		
Sample ID	Run #	$\delta^{18}O$, H_2O (‰, V-SMOW)	Mean ± SD
*Salt Lake City, UT ground H_2O (BSLC)	1	−15.34, −15.46, −15.70	−15.50 ± 0.09
	2	−15.52, −15.48	
	3	−15.53, −15.47	
	4	−15.49, −15.51	
*Laihaii, HI bottled H_2O (SHOW)	1	−3.28, −3.16, −2.85, −3.51	−3.20 ± 0.17
	2	−3.11, −3.29	
	3	−3.16, −3.24	
	4	−3.09, −3.31	
Carson, WA stream H_2O	1	−11.09	−11.20 ± 0.16
	2	−11.43	
	3	−11.20, −11.35, −11.12, −11.03	
University of Utah Zero (UUZero)	1	−0.78, −0.86	−0.63 ± 0.17
	2	−0.63, −0.32	
	3	−0.58, −0.65, −0.58	
Salt Lake City, UT tap H_2O	1	−16.34, −16.36, −16.44	−16.40 ± 0.06
	2	−16.48, −16.42, −16.45	
	3	−16.38, −16.32	
EVIAN® bottled H_2O	1	−10.12, −10.21, −10.26	−10.05 ± 0.16
	2	−9.91, −9.97, −10.14	
	3	−9.84, −9.91	

* Salt Lake ground water and Laihaii water measured as −15.50‰ and −3.20‰ by standard equilibration methods.

SMOW; laboratory working standard); (2) Salt Lake City UT tap water; (3) Laihaii, HI bottled water (with known $\delta^{18}O$ value of −3.20‰ relative to V-SMOW; laboratory working standard); (4) Evian® bottled water; (5) Carson, WA stream water; and (6) a mixture of 85% $H_2{}^{18}O$ and deionized water collected from the University of Utah, in Salt Lake City, UT. Each of the six water samples was subsampled into 6–13 equilibration vials for a total of 48 analyses. The 48 samples were measured over the course of four separate runs. Volumes of water used were in the range 40–100 μL. Table 1 presents results for the replicate analyses of each water sample after a 2-day equilibration period. Isotope ratio values of the two laboratory standards were statistically indistinguishable from values determined using a dual-inlet analysis (2-tailed t-test: $t = 1.92$, $p = 0.09$ for BSLC and $t = 0.19$, $p = 0.85$ for SHOW). The measurement precision across different analysis tests ranged from ±0.06 to 0.17‰ with an average precision from all four unknown water samples of ± 0.13‰.

Application use

We applied this technique modification to determine the diurnal variation in $\delta^{18}O$ of water samples from plant, soil, and atmosphere compartments in an old growth forest located in the Pacific Northwest (Gifford Pinchot National Forest, WA, USA). Water vapor, needle water, stem water, and soil water were collected from various positions in a 60-m tall canopy. The water was isolated from organic tissues and soils through distillation[8] and the isolated waters were equilibrated with dilute CO_2 (10% CO_2/90% N_2) and measured as described above. Sample sizes ranged from 80–1000 μL.

It is known that plants do not fractionate against ^{18}O during water uptake from the soil by roots and thus stem water should be reflective of the soils from where plants have extracted water. We observed that stem water $\delta^{18}O$ values (reflecting soil water) did not change with time or height in coniferous species (−8.3 ± 0.4‰), implying a constant water source (Fig. 4). These tree water $\delta^{18}O$ values differed from that of soil water near the surface ($\delta^{18}O = -6.1 \pm 0.5$‰), indicating that large trees were likely extracting water from below the surface soil layers. In contrast, the 1–2-m tall shrubs had stem water values of −5.5 ± 0.5‰, indicating that their water source was closer to the surface soil layers. Leaf or needle water becomes evaporatively enriched when stomata open allowing transpiration to occur, with the degree of leaf water enrichment related to the vapor pressure deficit. This pattern was clear from the predawn to midday changes in leaf/needle water changes. First, at predawn with a low vapor pressure deficit, leaf/needle water $\delta^{18}O$ values were nearly the same as their source water (stem waters). Second, the extent of evaporative enrichment was a function of the vapor pressure deficit that developed within the canopy. The lowest humidities were found at the tops of the canopy. Thus leaf water $\delta^{18}O$ values were most different from source waters at the top of the canopy and progressively closer to source waters at the bottom of the canopy. If the bulk of the water vapor was in equilibrium with source water, then the $\delta^{18}O$ values of water vapor should remain constant through the day and the values should be depleted by 8–10‰ from the stem water values. This is indeed what we measured. The $\delta^{18}O$ in water vapor did not change over the course of the day (column average = −14.2 ± 0.7‰ at predawn vs. −14.8 ± 0.7‰ at midday).

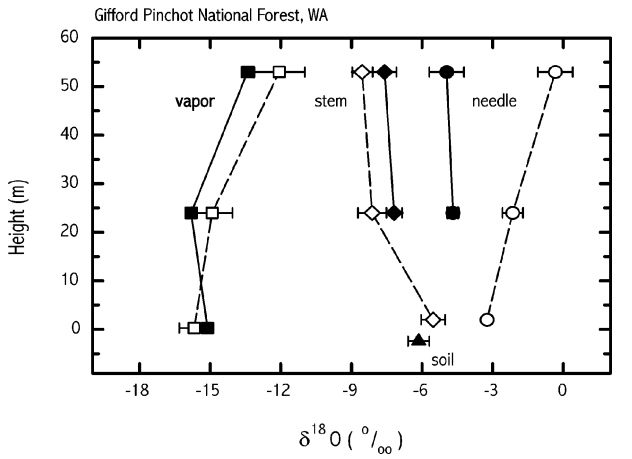

Figure 4. Variation of $\delta^{18}O$ in H_2O reservoirs found in a 500-year-old forest in the Gifford Pinchot National forest, WA, USA. Predawn (closed symbols) and midday (open symbols) collections of canopy water vapor (■), stem water (◆), and needle water (●) are displayed with comparisons made with soil water (▲ = soil water collected from soils up to 0.5 m). Error bars represent standard errors of six (soil and vapor) to nine (stems and needles) measurements. Predawn collections were made 1 h before sunrise and midday collection was taken at the height of photosynthesis (12:00).

CONCLUSIONS

The modifications to the CF-IRMS described allow for rapid processing rates and yet retain high-precision $\delta^{18}O$ values for dilute CO_2 concentrations equilibrated with small water volumes. Water volumes of 40–100 μL of H_2O can be analyzed for oxygen isotope ratio with a precision of ± 0.13‰ at a rate of 20 samples per hour. The modifications made to the mass spectrometer system have no effect on the normal use of the CF-IRMS system, thereby increasing the overall utility of the CF-IRMS.

Acknowledgements

We would like to thank C. F Kitty for laboratory assistance and Meghann Hunter for designing Fig. 1.

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