RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2004; **18**: 903–906
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/rcm.1426

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Isotopic (^{13}C) analysis of dissolved organic carbon in stream water using an elemental analyzer coupled to a stable isotope ratio mass spectrometer

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Received 13 November 2003; Revised 25 February 2004; Accepted 25 February 2004

A technique for measurement of the stable isotope composition of dissolved organic carbon (DOC) in stream water, using an elemental analyzer (EA) coupled to an isotope ratio mass spectrometer (IRMS), is described. Stream water samples were concentrated by rotary evaporation, acidified to remove dissolved inorganic carbon (DIC), and dried in silver cups prior to analysis. Precision was evaluated with standards (alanine and humic acid), and with stream water samples with varying ^{13}C enrichment. Standards and samples were also prepared in sealed quartz tubes for high-temperature combustion (HTC) and analyzed by dual inlet for comparison. The δ^{13}C values of natural abundance standards and samples measured by the two techniques differed by $\leq$0.3‰. Isotopically enriched stream water samples analyzed using the EA and HTC methods had comparable δ^{13}C and atom % values (differed by $\leq$6%). Expensive quartz vessels and the extensive time required for sample lyophilization, combustion and cryogenic separation of combustion products before isotopic analysis for the HTC method are eliminated with the EA method. Overall, the EA method is simpler, faster, and more economical than the HTC method, and it can be used for ^{13}C-enriched as well as natural abundance samples.

Dissolved organic carbon (DOC) is the largest pool of reduced carbon in aquatic ecosystems and an important energy source for heterotrophic bacteria.[1] Despite its prominence as a carbon reservoir, this pool is difficult to characterize chemically because it is a heterogeneous mixture of mostly unidentified molecules.[2] Methods for accurate isotopic analysis of the DOC pool have faced many challenges. Previous methods used to determine the isotopic composition of DOC include persulfate oxidation, high-temperature sealed-tube combustion (HTC) of lyophilized samples, direct sample injection into a high-temperature furnace, and a total organic carbon (TOC) analyzer coupled to a continuous flow isotope ratio mass spectrometer.[2–11] The HTC method has been identified as a potential reference method for DOC analysis because the oxidation of organic matter is quantitative.[9,10,12] However, this method requires expensive quartz vessels and extensive time to lyophilize and combust DOC samples before isotopic analysis. Here we describe a continuous flow technique to measure the isotopic composition of DOC in stream water using an elemental analyzer (EA) coupled to an isotope ratio mass spectrometer (IRMS). This EA method was calibrated against the HTC method. Data presented for natural abundance and enriched samples show that the EA and HTC methods produce comparable results. However, the EA method is faster and more cost-effective than HTC.

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EXPERIMENTAL

Sample preparation

Stream water samples were collected from the East Branch of White Clay Creek (WCC), a third-order stream located in the Piedmont Province of southeastern Pennsylvania, USA (39°53′N, 75°47′W). Samples were filtered through either a three-stage (75, 25, and 0.3 μm) glass fiber cartridge filter system (Ballston, Cleveland, OH, USA)[13] or pre-combusted (500°C, 6 h) GF/F (Whatman, Clifton, NJ, USA) filters, and stored frozen in 250-mL low-density polypropylene (Nalgene, Rochester, NY, USA) containers. Some stream water samples were amended with ^{13}C-labeled tree tissue leachate (DOC 120 mg C L^{-1} with δ^{13}C = +6160) at tracer levels that did not increase the stream DOC concentrations by more than 5%.[14]

Elemental analyzer method

Standards and stream water samples containing approximately 20 μmol C (equivalent to 150–200 mL of stream water) were concentrated in a round-bottomed flask by rotary evaporation to a volume of about 1 mL (±0.5 mL). Concentrated samples were acidified to a pH of 2 with 85% phosphoric acid

(ca. 100 μL) and agitated to remove DIC and transferred to a pre-combusted (500°C, 2 h) glass scintillation vial (Fisher, Pittsburgh, PA, USA). The flask was rinsed with two 1.5-mL aliquots of ultra-pure deionized water (Barnstead E-pure) to enhance quantitative transfer. The rinse resulted in a final volume of 3.5–4.5 mL. 300 μL of the concentrated sample were transferred to silver capsules (8 mm × 5 mm; EMAL, Mason, OH, USA) in three 100-μL aliquots. The samples were dried in an oven (70°C) for 1 h after each transfer. The three-step transfer was necessitated because of the low volume (ca. 100 μL) of the silver capsules. The dried samples were then loaded into the auto-sampler of an EA (EA3000, Eurovector, Milan, Italy) coupled to an IRMS (GV Instruments, Manchester, UK). The sample was flash combusted in a temporarily oxygen-enriched atmosphere of a combustion reactor (chromium oxide, cobaltous oxide, quartz chips, quartz wool; EMAL) held at 1030°C. The oxidation products were carried by a stream of helium through a reduction reactor (copper, quartz chips, quartz wool; EMAL) at 650°C. The resulting gases (primarily CO_2, N_2 and H_2O) were then carried through a magnesium perchlorate filter to remove water. The CO_2 and N_2 were separated in a chromatographic column (3 m, packed column), passed through a thermal conductivity detector, and carried into the source of the IRMS where the isotope ratios were measured against a pulse of reference gas of known isotopic composition. The carbon yield was estimated with a standard of known carbon content (glycine, Sigma, St. Louis, MO, USA) and calibration achieved from a regression of peak height (mass 44) versus the carbon content of the standard. Isotope ratios are expressed in per mil:

$$\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (1)$$

where R is the abundance ratio of ^{13}C to ^{12}C and the standard is V-PDB.[15]

High-temperature sealed-tube combustion method

Samples were analyzed using a HTC method modified after Feuerstein *et al*.[10] for the analysis of dissolved organic nitrogen (DON) and DOC. Briefly, samples were concentrated to 1–2 mL using rotary evaporation, acidified and transferred to a pre-combusted (500°C, 2 h) quartz vessel (220 mm × 12 mm) with an enlarged bulb on the terminal end (50 mm o.d.). Samples were lyophilized on a glass vacuum drying line and, upon dryness, 2 g of combusted CuO (850°C, 4 h; EMAL), 6 g of combusted pure Cu granules (850°C, 4 h; EMAL) and 0.5 g of Cu turnings (EM Science, Gibbstown, NJ, USA) were placed in the neck of the vessel above a quartz wool plug. Samples were re-evacuated and sealed under vacuum at <5 mTorr. Sealed samples were shaken and combusted following a modified Dumas combustion procedure.[16] After combustion, sample vessels were scored while hot (100°C), cracked open under high vacuum, and the CO_2 cryogenically isolated. The purified CO_2 was analyzed on a PRISM (GV Instruments, Manchester, UK) IRMS. Carbon yield was measured with a Baratron capacitance manometer (MKS Instruments, Andover, MA, USA) during cryogenic separation. The Baratron was calibrated with known volumes of pure CO_2.

Standards and inter-laboratory calibration

Two standards (alanine, Sigma; humic acid, International Humic Substance Society, St. Paul, MN, USA) containing 25 μmol C were prepared in 200 mL of ultra-pure deionized water and processed for analysis like the stream water samples. One of the stream water samples was also used for an inter-laboratory calibration with the G.G. Hatch Isotope Laboratories (Ottawa, Canada) where it was analyzed by the method of St-Jean[11] using a TOC analyzer coupled to an IRMS.

Carbon recovery

Two approaches were used to determine carbon recovery for each of our combustion methods. First, we compared our empirically derived carbon content of standards with their known values. Second, we compared the carbon content of the stream water samples determined by our methods with those from a TOC analyzer (either an OI 700 or an OI 1010; O.I. Corp., College Station, TX, USA).[17]

RESULTS AND DISCUSSION

Our objective was to develop a simplified, rapid, and economical method for the analysis of ^{13}C DOC that would be suitable for ^{13}C tracer experiments at the ecosystem scale. Ecosystem additions of isotopically enriched molecules, in our case ^{13}C DOC, enable sources and transformations of dissolved and particulate materials to be elucidated and *in situ* rates of chemical and biological processes to be determined.[18] In our study, the introduction of DOC with high ^{13}C-enrichment levels resulted in negligible increases in the ambient stream DOC concentration, and thus avoided artificially stimulating biological activity of the stream biota. Because only small amounts can be added, the tracer must be highly enriched in the rare isotope. Thus, one must be able to measure high levels of enrichment to characterize the initial tracer and subsequent samples into which it is incorporated. Consequently, we required a method to analyze both levels at natural abundance and ^{13}C-enriched samples.

Previously, we used the method of Feuerstein *et al*.[10] to measure DON and DOC isotope values of lacustrine waters. This method involves sample concentration via rotary evaporation, dialysis to remove inorganic nitrogen, static combustion in sealed quartz tubes, cryogenic purification, and isotopic analysis using the dual inlet of an IRMS. However, relative to many lacustrine waters, the potentially high dissolved ion concentrations in some streams may pose a problem for DOC recovery. Fry *et al*. showed that, in the presence of salts, DOC samples can slowly lose more than 90% of the CO_2 from the gas phase through sorption.[8] To minimize CO_2 sorption for samples prepared by HTC, we initiated cryogenic separation of our samples before they cooled below 100°C. Our approach yielded high carbon recoveries for standards (>97%) and stream water samples (83–88%) and demonstrated that CO_2 sorption can be minimized and isotopic fractionation avoided (Table 1). However, the HTC method is expensive and time consuming. The primary cost is the quartz vessels ($25 per vessel). Excessive

Table 1. Comparison of elemental analyzer (EA) and high-temperature sealed-tube combustion (HTC) analyses for ^{13}C and carbon recoveries for alanine, humic acid, and three stream water samples. Differences between methods are reported as Δ

	C Recovery (%)			$\delta^{13}C_{PDB}$ (‰)		
Analyte	EA	HTC	Δ	EA	HTC	Δ
Alanine	96.0 ± 0.9[a]	99.5	3.5	−23.3 ± 0.1	−23.6	0.3
Humic acid	94.7 ± 3.2	97.1	2.4	−26.7 ± 0.1	−26.8	0.1
Stream water-1*	65.4 ± 2.8	88 ± 5.3	22.6	−26.8 ± 0.2	−26.8 ± 0.1	0.0
Stream water-2	74.5 ± 3.8	83.4	8.9	−26.6 ± 0.0	−26.9	0.3
Stream water-3	64.0 ± 1.1	84.3	20.3	−26.0 ± 0.1	−26.0	0.0

* The value for this sample determined by a TOC analyzer interfaced to an IRMS in the G.G. Hatch Isotope Laboratories was $\delta^{13}C = -26.8 \pm 0.2$ (n = 3).
[a] Data reported as x ± S.D., n = 3 for EA, and n = 1 for HTC, except *, where n = 3.

time associated with lyophilization (15–20 h on the drying line), combustion (24 h) and cryogenic separation of samples (20 min per sample) limits the number of samples that can be processed to five per week. Hence, this technique is impractical for large numbers of samples.[8,10]

Given the cost and effort associated with the HTC method, we explored using an elemental analyzer (EA) interfaced to an isotope ratio mass spectrometer (IRMS) for isotopic analysis of DOC. The flash combustion and short analysis time in this method minimize the opportunity for evolved CO_2 to react with salts. To accommodate the low volume (ca. 100 μL) of the silver capsules, 300 μL of the concentrated sample were transferred in three 100-μL aliquots using a Finpipette pipette (40–200 μL, Fisher). Carbon recovery estimates using the EA method ranged from ca. 95% for standards to 64–75% for stream water samples (Table 1). For these stream water samples, a separate pipette tip was used to transfer each of the three 100-μL aliquots to the silver cup. We believe that the low recovery for these samples reflected sorption of sample on the pipette tip rather than a problem with combustion. In subsequent analyses, a single pipette tip was used to transfer the three 100-μL aliquots, and recoveries increased to 75% or greater. Notably, our evaluation of accuracy shows that isotopic integrity appears to be maintained during the EA combustion method.

The accuracy of $\delta^{13}C$ values obtained by the EA method was compared with those from the HTC method using both standards (alanine or humic acid) and stream water samples at natural abundance and enriched levels (Tables 1 and 2). The differences between the EA and HTC values were 0.3‰ or less. A paired t-test showed no statistical differences among the $\delta^{13}C$ values obtained for the natural abundance samples by the EA and the HTC methods (Table 1, $t(4) = 2.06$, $p = 0.11$). The inter-laboratory comparison showed that the $\delta^{13}C$ values from the EA and HTC methods were identical to those produced by an independent method described by St-Jean[11] (TOC analyzer coupled to an IRMS) at the G.G. Hatch Isotope Laboratories, Ottawa (Table 1). A paired t-test showed no statistical differences among the atom % values obtained by EA and HTC analysis of enriched stream water samples (Table 2, $t(3) = 0.99$, $p = 0.39$). The difference in the results from the EA and HTC methods for stream water samples with varying levels of enrichment was less than 6%. These results, in conjunction with our previous findings, demonstrate that the EA method can be used to examine stream DOC isotopic composition at both natural abundance and enriched levels.

CONCLUSIONS

Here, we present a new technique to measure the isotopic composition of DOC in stream water using an EA coupled to an IRMS. This new method gives results comparable to those from our HTC method, yet in our laboratory provides for an eight-fold higher sample throughput at a lower cost. The time required to concentrate the stream water is the same for both techniques. However, after concentration, the samples can be dried and analyzed by the EA-IRMS system the same day, whereas for the HTC method samples must be transferred to the quartz vessel, lyophilized (15–20 h),

Table 2. Comparison of atom % values for four ^{13}C-enriched samples analyzed with elemental analyzer (EA) and high-temperature sealed-tube combustion (HTC) methods. Differences between methods are reported as Δ

	Atom %		
Sample	EA	HTC	Δ
Stream water amended with ^{13}C tree tissue leachate-1	1.1047 ± 0.0002[a]	1.1044	0.0003
Stream water amended with ^{13}C tree tissue leachate-2	1.1577 ± 0.0036	1.1667	0.0090
Stream water amended with ^{13}C tree tissue leachate-3	1.2788 ± 0.0043	1.2706	0.0082
Stream water amended with ^{13}C tree tissue leachate-4	1.7076 ± 0.0104	1.7876	0.0800

[a] Data reported as x ± S.D., n = 3 for EA, and n = 1 for HTC.

combusted (24 h) and cryogenically separated on a vacuum line (20 min per sample), before isotopic analysis. When compared with the HTC technique, the analysis by the EA-IRMS method is relatively simple, eliminating the high cost of quartz vessels and lengthy times required for lyophilization, combustion and cryogenic separation. Weekly, five samples could be analyzed by the HTC method in our laboratory; however, with the EA technique, we are able to analyze up to 40 samples per week. Our results suggest that the EA technique can be effectively used for routine isotopic analysis of natural abundance and ^{13}C-enriched DOC in stream water samples. This provides a significant new opportunity for many laboratories that already retain an EA interface to their IRMS.

Acknowledgements

We would like to thank Paul Middlestead of the G.G. Hatch Isotope Laboratories for analyzing the stream water sample for inter-laboratory comparison. This work was supported by NSF grant DEB-0109122 to LAK and PHO and NSF DEB-0096276 to LAK.

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