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ARTICLE *in* ANALYTICAL CHEMISTRY · JULY 2008

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Technical Notes

Coupling a High-Temperature Catalytic Oxidation Total Organic Carbon Analyzer to an Isotope Ratio Mass Spectrometer To Measure Natural-Abundance δ^{13}C-Dissolved Organic Carbon in Marine and Freshwater Samples

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The stable isotope composition of dissolved organic carbon (δ^{13}C-DOC) provides powerful information toward understanding carbon sources and cycling, but analytical limitations have precluded its routine measurement in natural samples. Recent interfacing of wet oxidation-based dissolved organic carbon analyzers and isotope ratio mass spectrometers has simplified the measurement of δ^{13}C-DOC in freshwaters, but the analysis of salty estuarine/marine samples still proves difficult. Here we describe the coupling of the more widespread high-temperature catalytic oxidation-based total organic carbon analyzer to an isotope ratio mass spectrometer (HTC-IRMS) through cryogenic trapping of analyte gases exiting the HTC analyzer for routine analysis of δ^{13}C-DOC in aquatic and marine samples. Targeted elimination of major sources of background CO_2 originating from the HTC analyzer allows for the routine measurement of samples over the natural range of DOC concentrations (from 40 μM to over 2000 μM), and salinities (<0.1−36 g/kg). Because consensus reference natural samples for δ^{13}C-DOC do not exist, method validation was carried out with water-soluble stable isotope standards as well as previously measured natural samples (IAEA sucrose, Suwannee River Fulvic Acids, Deep Sargasso Sea consensus reference material, and St. Lawrence River water) and result in excellent δ^{13}C-DOC accuracy (±0.2‰) and precision (±0.3‰).

Measurement of the stable isotope composition of carbon (δ^{13}C) has proven a powerful approach for deciphering the sources and cycling of dissolved organic carbon (DOC) in freshwater and marine systems.[1–6] The methods employed to measure δ^{13}C-DOC in marine samples tend to be long and tedious usually requiring highly specialized, expensive equipment, large sample volumes, and a number of steps to remove carbonates and quantitatively convert DOC into CO_2 (by UV oxidation or combustion in an O_2 atmosphere) for eventual off-line analysis by mass spectrometry.[7,8] Alternatively, concentrating a sample (by lyophilization or evaporation) to obtain δ^{13}C-DOC by solid-state elemental analysis coupled to an isotope ratio mass spectrometer (EA-IRMS) is a frequently chosen option. This approach works well when the ratio of DOC to inorganic salt is relatively high;[9] however, the low DOC to salt ratios that characterize estuarine/marine samples introduce a high degree of uncertainty and poor reproducibility in the measurement,[10] limiting this approach to freshwaters. Considering the importance of coastal zones to the general health of the global ocean as well as the significant role they play in the global economy, understanding how coastal phenomena are related to the carbon cycle is of growing importance.

Generally δ^{13}C-DOC is more depleted in freshwater systems (−26 to −30‰) and more enriched toward marine sites (−22 to −20‰) and the signature in the open ocean is relatively invariant with depth or location.[6] δ^{13}C-DOC mixing behavior between the fresh and marine end-members in estuaries and coasts has been modeled,[4] and when unperturbed, most systems can be predicted to a good degree. However when a coastal zone or estuary has been perturbed due to either climate change or land-use changes, the organic carbon dynamics may also be altered in a number of

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10.1021/ac702641z CCC: $40.75
Published on Web 06/05/2008

ways that are difficult to detect by current remote sensing techniques or by simple DOC quantitation. Monitoring these alterations allows for a better understanding of carbon biogeochemical cycling and the extent to which the ecosystem as a whole is affected. δ^{13}C-DOC is at the same time one of the most effective and potentially the simplest approach to interpret these processes. Though δ^{13}C-DOC can provide highly informative data toward the understanding of dissolved carbon dynamics, analytical limitations have precluded its routine measurement in estuarine, coastal, and marine systems.

The direct coupling of wet oxidation total organic carbon (TOC) analyzers to IRMS instruments (WO-IRMS) has recently been reported[10–12] and greatly simplifies the measurement of δ^{13}C-DOC to a high degree of accuracy and precision, particularly for freshwater samples. In this setup, a volume of water is injected into a reaction vessel containing a chemical oxidant (e.g., sodium persulfate) or exposed to UV light to oxidize DOC to CO_2. The sample flow is then directed through halogen scrubbers and chemical reductants typically used in EA-IRMS instrumentation and finally to the mass analyzer. However, the large sample volumes required for a typical WO-IRMS analysis of marine DOC (up to 25 mL[11]) preclude its use for volume-limited samples, such as surface sediment marine porewaters. By modifying a WO-IRMS system to be more amenable to marine samples through adjustments made to the WO system and improving IRMS sensitivity, accurate and reproducible measurement of as little as 2 mL of a coastal marine sample was achieved.[12] The authors note however that following as few as 10 sample injections (~2.5 h of analysis time), halide gases produced from the oxidation conditions as well as salt deposition in the flow lines of this system lead to rapid corrosion of some parts of the reaction vessel, fouling of the halide trap, and exhaustion of the reducing agents, in addition to flow restriction in the lines.

In a recent interlaboratory DOC analysis comparison study, 61 of 68 participants reported results with high-temperature catalytic oxidation (HTC) TOC analyzers, reflecting their use as the overwhelming tool of choice for quantitative DOC analysis.[13] In these instruments, a small volume of sample (50−150 μL) is injected over a catalyst capable of generating oxygen radicals (such as Pt-impregnated alumina[14] or silica rods[15]) at high temperature to quantitatively convert DOC to CO_2, which is then detected by nondispersive infrared (NDIR) spectrophotometry. One advantage of HTC is the low volumes needed for analysis compared to wet oxidation (0.050−0.200 vs 2−25 mL per injection), which allow running more replicates from smaller sample volumes. Because of the popularity and convenience of HTC-based analyzers compared to the WO variety, some groups have reported efforts to couple them to IRMS instruments.[16–18] The hyphenated system generally involves trapping, either with sorbents[16] or, cryogenically, with liquid N_2,[17,18] the CO_2 in the stream of carrier gas as it exits the TOC, then directing the flow (on- or off-line) to an EA-IRMS system for δ^{13}C determination. In an off-line cryogenic trapping system in which trapped sample gases are introduced into the combustion column of an EA-IRMS, the accurate determination of the δ^{13}C-DOC of deepwater marine samples (~44 μM C) was made possible but required 2−3 h of analysis time and up to 20 mL per sample.[17] Applications of on-line HTC-IRMS analysis for δ^{13}C-DOC are generally suited to high-concentration DOC samples such as sediment porewaters[16] or interstitial soil DOC.[18] HTC-IRMS has also been designed to determine the δ^{15}N of total dissolved nitrogen (TDN)[19] but again detection limits are poor and are at least a full order of magnitude higher than what is typical of natural systems.

Here we describe an on-line HTC-IRMS system for the simultaneous quantitative and stable isotope measurement of DOC in natural (marine and freshwater) samples using a Shimadzu TOC 5000A instrument, one of the more widely used TOC analyzers.[13] The overall approach consists of cryogenically trapping gas flowing from the TOC analyzer, then directing the flow to the reduction and gas chromatography columns of an EA-IRMS system, and finally toward the IRMS in a setup similar to one used for the analysis of δ^{15}N-TDN.[19] In the system described here, comparatively fewer major modifications to the HTC analyzer are needed and a much less expensive, more available catalyst that allows pure He to be used as a carrier gas is employed. Additionally, cryogenic trapping of combustion gases carry the advantage of focusing CO_2 resulting in sharper IRMS peaks. Superior detection limits to other on-line HTC-based TOC-IRMS systems are obtained owing to the systematic elimination of most sources of background CO_2 and open the door for routine δ^{13}C-DOC analysis of marine waters.

EXPERIMENTAL SECTION

Reagents and Standards. IAEA-CH-6 certified sucrose standard (International Atomic Energy Agency, −10.45 ± 0.03‰),[20] Suwannee River Fulvic Acid standard (SRFA, International Humic Substances Society, −27.6 ± 0.12‰), and β-alanine (Sigma-Aldrich, −26.18 ± 0.33‰ standardized in-house against several certified materials by EA-IRMS) were used as reference δ^{13}C-DOC compounds. Potassium hydrogen phthalate (KHP) was obtained from Shimadzu and is the conventional quantitative DOC standard. Unless otherwise noted, standard solutions were prepared in ultrapure water (Millipore Simplicity 185 equipped with a Simpak1 cartridge) over a wide range of concentrations (30−1050 μM C), acidified with 1 drop of TraceSELECT grade HCl (SigmaAldrich), and prepared regularly. Standard additions of SRFA to marine waters was carried out (see Supporting Information for experimental details). In addition, low-carbon (44−47 μM C) deep Sargasso Sea consensus reference material (DSS-CRM, Lot No. 00−12, D. Hansell, U. Miami), routinely used for validation and quality control of TOC instruments[21,22] was obtained to validate

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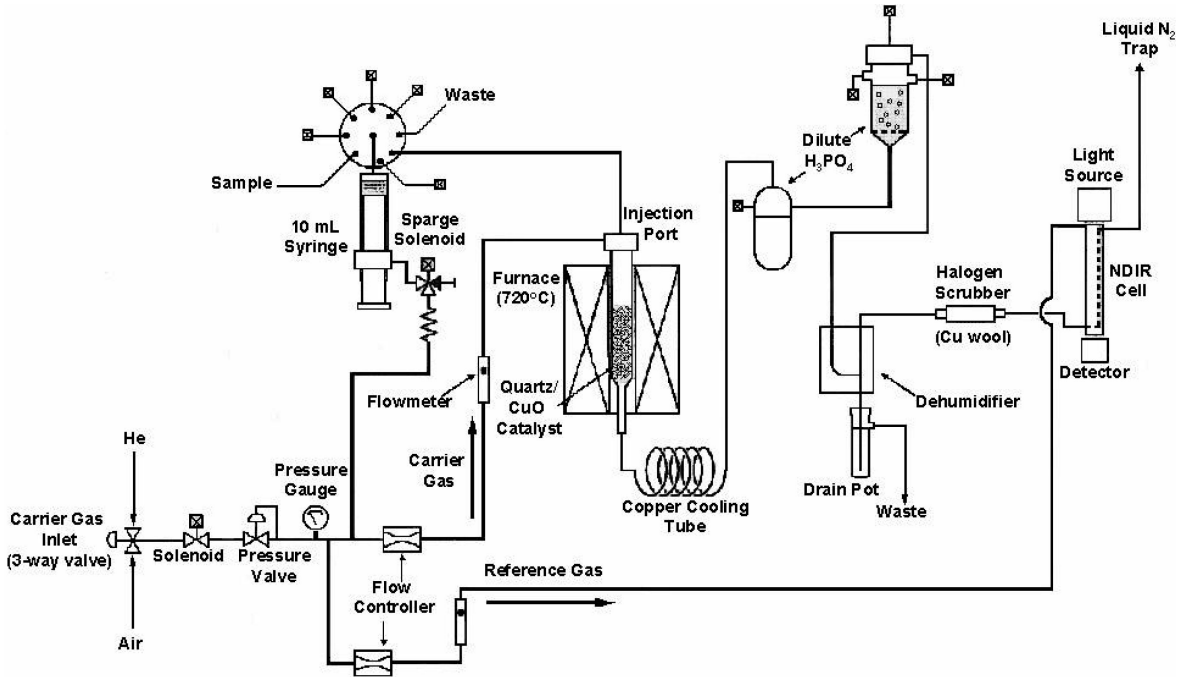

Figure 1. Schematic diagram of the modified Shimadzu TOC-V_{CPH} used in this study.

the system for δ^{13}C-DOC in natural waters. Several end-member natural samples were chosen to reflect salinity and DOC concentration extremes expected in the analysis of coastal samples (see Supporting Information for sample details). Conventionally, marine DOC concentrations are given in terms of micromolar elemental C, whereas EA-IRMS measurements are usually expressed in terms of mass; thus, to maintain consistency, all concentrations and mass concerning DOC or analyte CO_2 are expressed as moles of elemental C.

Total Organic Carbon Analyzer. The Shimadzu TOC-V_{CPH} 5000A total organic carbon analyzer, with external sparge gas option was used. The original tubing on the instrument consisted of $^1/_8$-in.-o.d. PTFE. Samples are drawn (4−6 mL, acidified to pH ≤2 with 6 N HCl) and sparged with carrier gas for 2−5 min, to remove dissolved inorganic carbon (DIC) species, i.e., carbonates. After DIC removal, 50−150 μL are injected over 2-mm Pt-impregnated silica spheres (Shimadzu) heated to 720 °C in a 2.8 × 30 cm (12-cm hot zone) quartz tube, where quantitative oxidation of DOC to CO_2 occurs. The combustion gases are directed through a $^1/_8$-in.-o.d. Cu cooling tube, a pure water trap, to trap volatile inorganics, the DIC reaction vessel, a dehumidifier, a Cu−wool halogen trap, and a 0.2-μm aerosol filter, before NDIR spectrophotometric detection of CO_2. The carrier gas is then stripped of CO_2 by a soda lime scrubber and recycled as CO_2-free reference gas for NDIR.

Total Organic Carbon Analyzer Modifications. Figure 1 shows the schematic of the modified instrument. First, IRMS requires high-purity He to be used as carrier gas, a viable option when the original catalyst (Pt-impregnated silica spheres) is used.[23] However, this catalyst was found to be a very significant contributor to background CO_2 (see Results section) and was replaced with 2 × 2 mm quartz rods mixed with cupric oxide[15] (~60:40 mass ratio); thus, the catalyst itself is an oxidant in the combustion chamber. The amount of CuO should not exceed this ratio, and care should be taken to ensure homogeneity of the catalyst mixture to avoid combustion column cracking when cooled. A three-way valve was installed to allow the user to switch between air and He as carrier gas, depending on whether the instrument is used for TOC (NDIR quantitation only) or TOC-IRMS (NDIR quantitation and δ^{13}C-DOC). The carrier gas is also switched to air overnight to regenerate the cupric oxide when oxidation efficiency becomes inconsistent (e.g., when the standard deviation for the analysis of quantitative standards exceeds ±5%, typically after ~300 natural sample injections). A 1-cm-thick layer of cobaltous silver oxide was also added at the bottom of the column as a primary halogen trap.

It has also been recognized that the PTFE tubing used in DOC analyzers is permeable to atmospheric CO_2,[17] and with the exception of the sampling tube, all were replaced with $^1/_8$-in.-o.d. stainless steel tubing, while retaining the original fittings. Other polymeric tubing materials were considered, such as PEEK or ECTFE, both materials with CO_2 permeabilities significantly lower than PTFE; however, permeation with these materials would still occur, as opposed to stainless steel for which gas permeability is zero. In parts where metric-sized tubes and fittings are used (such as pressure valves), the original PTFE was used as a sleeve over the end of the $^1/_8$-in. stainless steel tubing.

Several drops of reagent-grade 85% phosphoric acid were added to ultrapure water in the water trap to prevent CO_2 dissolution in the system, thus reducing sample carryover. The only necessary modification to the Pelletier dehumidifying unit was the replacement of the $^1/_2$-in.-o.d. plastic waste tube with $^1/_8$-in.-o.d. polypropylene tubing to prevent increased flow out of the unit, which sometimes occurred when trapping high DOC concentration solutions. Attempts were made at bypassing components of the system that are superfluous when used in the TOC or TOC-IRMS modes (e.g., the DIC reaction vessel), but the result was poor peak shape and varying retention time that adversely affected peak

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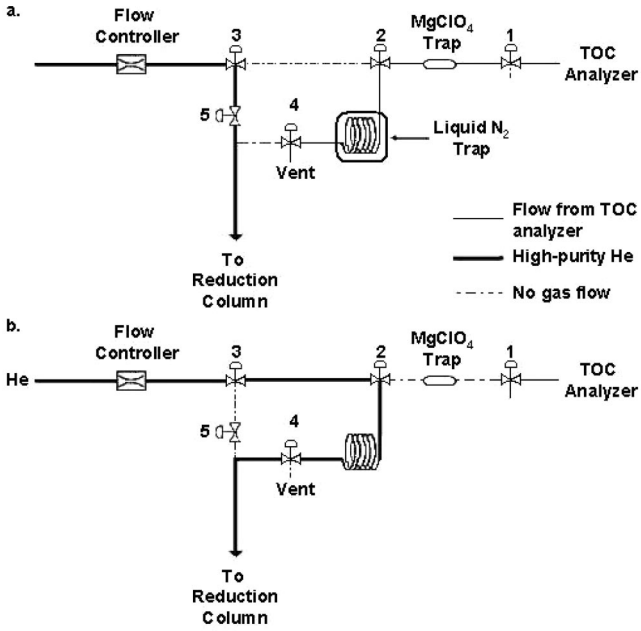

Figure 2. Schematic of the trapping loop and valve system in (a) trapping and (b) release configurations. To switch from trapping to release, first valve 2 is switched, followed a few moments later by valve 4 and valve 5, and valve 1 follows to vent the TOC. To return the system to the trapping mode, first valve 5 is switched, followed by valve 4, and then valves 1 and 2 simultaneously. This sequence is followed to prevent ambient CO_2 from entering the system, ensure a constant supply of carrier gas to the IRMS, and avoid pressure build-up in the TOC.

integration of the NDIR signal (data not shown).

Finally, carrier gas exiting the NDIR is redirected away from the CO_2 scrubber and toward a $Mg(ClO_4)_2$ water trap just prior to the cryogenic trapping loop. The external sparge gas option available on the TOC-V_{CPH} is used to provide a flow of CO_2-free gas for the NDIR reference allowing quantitation of TOC.

Cryogenic Trapping, Chemical Reduction, and Chromatographic Separation of TOC Gases. The trapping loop is a 1.5-m-long, $^1/_8$-in.-o.d. stainless steel tube set in a 10-cm-diameter coil. To trap CO_2 exiting the TOC, the coil is immersed in a bath of liquid nitrogen (LN). The pressure from the TOC is insufficient to force gas flow through the gas chromatography column of the EA-IRMS; thus, a series of valves was set so that the flow from the TOC and the flow toward the EA system do not simultaneously overlap (Figure 2), a situation that would cause overpressure in the TOC, and loss of flow to the IRMS, resulting in the introduction of ambient air to the ion source of the IRMS.

Trapped sample gases produced from combustion (mainly CO_2, and various NO_x) are released by removing the loop from the LN trap. The gas flow is introduced into the reduction column (quartz tube packed with elemental Cu heated to 680 °C) of a GV Instruments EuroVector IsoPrime EA-IRMS system under control of MassLynx 4.0 software (Manchester, UK) to chemically reduce the NO_x species present in the sample gas to N_2. Interference from some NO_x species, in particular N_2O ($m/z = 44$) with the measurement of CO_2 necessitates this step.[24] A layer of cobaltous silver oxide placed at the beginning of the Cu reduction column acts as a third halogen trap. After passing through a second $Mg(ClO_4)_2$ water trap, gases are separated in the regular GC column (set at 40 °C) of the EA system and transferred to the IRMS.

Isotope Ratio Mass Spectrometer. The ion source parameters are fine-tuned for carbon regularly and vary within normal limits (see Supporting Information for details). Research grade CO_2 (Praxair) is used as monitoring gas and set to match the expected intensity of trapped sample gas, ~1 nA (as major height). Baseline signal of ^{13}C with this instrument is typically $<10^{-4}$ nA resulting in signal-to-noise ratios of 12 for the lowest sample intensities (Figure S-1). Because peak intensity of natural samples is low and consequently in a linear dynamic range different from that typically used for this instrument, trials were conducted with the trap current set at higher values (up to 400 μA), but the gain in sensitivity is offset by the severe loss of dynamic range (data not shown). Thus, to maintain consistency and avoid overlapping linear dynamic ranges, peak heights for natural samples and dissolved standard compounds between 0.2 and 1.5 nA were targeted during routine analyses (Table S-1, Figure S-2). Reproducibility of the monitoring gas was typically within ±0.01 and 0.05‰ in a given day.

δ^{13}C-DOC Measurement. For δ^{13}C-DOC analysis, the trap setup is initially configured as in Figure 2a. The TOC instrument first draws 1 mL of the acidified sample to rinse the syringe, followed by an additional 6 mL of sample, which is then sparged of DIC for 6−10 min with He (shorter sparge times typically used for NDIR detection proved insufficient for the more sensitive IRMS). Six 150-μL injections are required to reach IRMS detection limits in marine samples (i.e., <80 μM C), and samples above 200 μM C can be accurately determined with only two traps. For consistency in background correction of δ^{13}C-DOC data, it is prudent to keep the number of traps equal for all samples (see Results and Discussion). The sample loop is immersed in LN immediately following the first injection. In some cases (following standard compounds, or the first sample of the day), two 150-μL samples are injected and not trapped, acting as a system rinse, purging the TOC of carryover from previous samples. When all injections are complete, the valves are configured as in Figure 2b, the sample loop is removed from the LN trap, and the isotope analysis using the IRMS software is launched. Following this sequence resulted in reproducible retention times as detected by the IRMS (265 ± 2 s, $n = 27$ over a two-day span). Immediately following the IRMS run, the trap lines are reconfigured as in Figure 2a. Analysis time ranges between 38 and 44 min for low- to high-concentration DOC samples trapped six times, respectively (10-min sparge, 22−28 min of trapping, 6 min for IRMS analysis). When a series of samples is being analyzed, time can be saved by sparging the next sample while the first sample is detected by IRMS, so that it takes ~30 min per sample, or 16 samples in an 8-h work day.

Data Analysis. Calibration and regression analysis is carried out using MATLAB (The Mathworks, Natick, MA, Version 7.0, Release 14). Total least-squares was used when two measured quantities were correlated (e.g., δ^{13}C and NDIR signal area). Data were corrected for TOC background as described in the next section.

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Table 1. Analytical Performance of the Modified TOC Analyzer

characteristic	before modification[a]	after modification[b]
retention time (minutes)	1.05	1.05
peak width (minutes)	1.4	1.4
precision (%)	1.9	1.6
accuracy (%)	1.4	0.27
response factor (mV·min/mmol C)	1.055	1.082
Stn 23 330m (μM C)[c]	62.8 (±1.3, $n = 6$)	64.2 (±0.9, $n = 16$)

[a] KHP standard solutions (40−800 μM C, $n = 43$) during routine analyses of marine and estuarine water samples spanning one month, fall 2006 using air as carrier gas. [b] β-Alanine, IAEA-CH-6, and KHP standard solutions (30−900 μM C, $n = 345$), Aug−Nov 2007) with high-purity He as carrier gas. [c] St. Lawrence Estuary, sampled June 2006.

RESULTS AND DISCUSSION

A direct coupling of the TOC analyzer to the IRMS inlet with only a halogen trap between the two was first carried out with IAEA-CH-6 and β-alanine dissolved in pure water to investigate the feasibility of hyphenating the two instruments for δ^{13}C-DOC measurements. These experiments resulted in acceptable IRMS peak shape (Figures S-1(b) and S-4) and also demonstrated no discernible isotopic fractionation during combustion in the TOC analyzer (−10.89 ± 1.0‰). However, the lower detection limit of the setup was only ~1500 μM C for IAEA-CH-6, which is inadequate for most natural waters (typically 40−400 μM C in the water column and up to 1200 μM C in sediment porewaters). As injection of larger volumes would lead to overpressure and possible cracking of the combustion tube of this TOC system, cryogenic trapping with LN after NDIR detection was set up to increase the amount of analyte CO_2 directed to the IRMS (described above). Hyphenation of the unmodified TOC to the IRMS via the reduction column of the EA system resulted in acceptable accuracy (after blank correction) of β-alanine solutions down to concentrations of 105 μM C with eight traps (−26.46 ± 0.90‰, $n = 4$). In order to improve both the precision and detection limits to encompass the full range of DOC concentrations found in natural systems, modifications targeting CO_2 permeation through the TOC were carried out.

Performance Characteristics of the Modified TOC Analyzer. The modifications carried out on the Shimadzu TOC-V_{CPH} improved accuracy and precision without adversely affecting any other performance characteristics (Table 1). In addition, the combustion efficiency of the silica rods with cupric oxide using high-purity He as a carrier gas is equivalent to the more expensive Pt-based catalyst, demonstrating the use of an oxygen pulse or He/O_2 carrier gas mixtures for analyses of natural DOC samples is not necessary. Background CO_2 released from the TOC (determined by IRMS) decreased ~2.3-fold following the described modifications. DSS-CRM was measured as 46.5 ± 2.6 μM C ($n = 18$) with the modified TOC, in agreement with what has been reported for this lot (D. Hansell, U. Miami. Personal communication 2007). In addition, multiple consecutive injections of a natural and sucrose-spiked freshwater sample collected from the St. Lawrence River near Québec City in June 2006 show no consistent trend in the measured concentration over these consecutive injections indicating that combustion efficiency remains unaffected and carryover is not significant, as previously

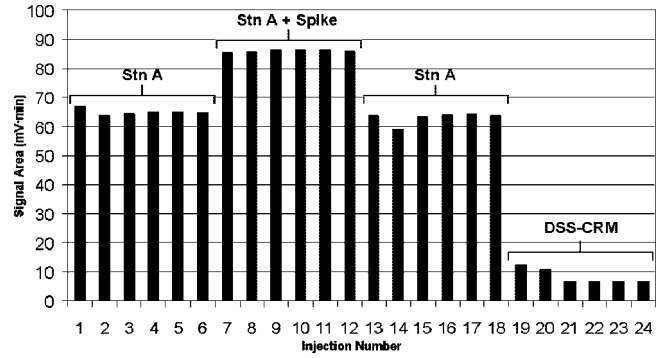

Figure 3. Demonstration of combustion efficiency and carryover characteristics of the catalyst system with He as carrier gas. Six injections of a natural freshwater sample from the St. Lawrence River (denoted Stn A, 424.8 μM) followed by six injections of the same sample spiked with sucrose (578.6 μM), six injections of Stn A, followed by six injections of DSS-CRM (45.1 μM). Only the first two injections of DSS-CRM (19 and 20) show evidence of carryover.

demonstrated for a silica rod-based catalyst system.[15] Analysis of DSS-CRM following these freshwater sample injections confirms this result (Figure 3). When catalyst regeneration with air was necessary, it would usually take two days of ultrapure water blank injections with He carrier gas to purge the system and return the background to acceptable levels for HTC-IRMS analysis of low-DOC marine samples.

***δ^{13}*C-DOC Determination: Background Contribution and Standard Solution Measurement.** As was discovered with DOC quantitation over a decade ago, background signal determination largely controls accuracy and precision of isotopic analysis by TOC-IRMS. All TOC systems, regardless of oxidation mode, are characterized by relatively high background CO_2 levels, originating from desorption of CO_2 from surface-active catalyst in the case of HTC analyzers and outgassing/contamination of reagents in the case of WO analyzers[25] or leaching of carbon from mechanical components,[26] including CO_2 permeation through plastic and PTFE tubing.[17] Because direct measurement of background is not possible with WO-IRMS systems developed to date, blank signals have been calculated through unsupervised iterative nonlinear optimization techniques[11,12] with blank defined as the value resulting in the least variation of the corrected sample δ^{13}C-DOC. This approach results in a highly variable background δ^{13}C (from −12 to −20‰),[11] which seems unlikely since the source of background CO_2 is relatively stable.[25,26] The calculated variability can be reduced by forcing the precision of the calculated results to match experimental precision and to repeat the iterative calculation several times to ensure false computational results are rejected. An advantage to this HTC-IRMS system over WO-IRMS is the ability to directly measure the blank signal, allowing experimental validation of background correction procedures, whether they are based on an iterative calculation approach or standard addition experiments.

Calibration with δ^{13}C-DOC standards shows that the relative blank contribution to the total signal intensity was strongly correlated to the concentration and not to the absolute mass of

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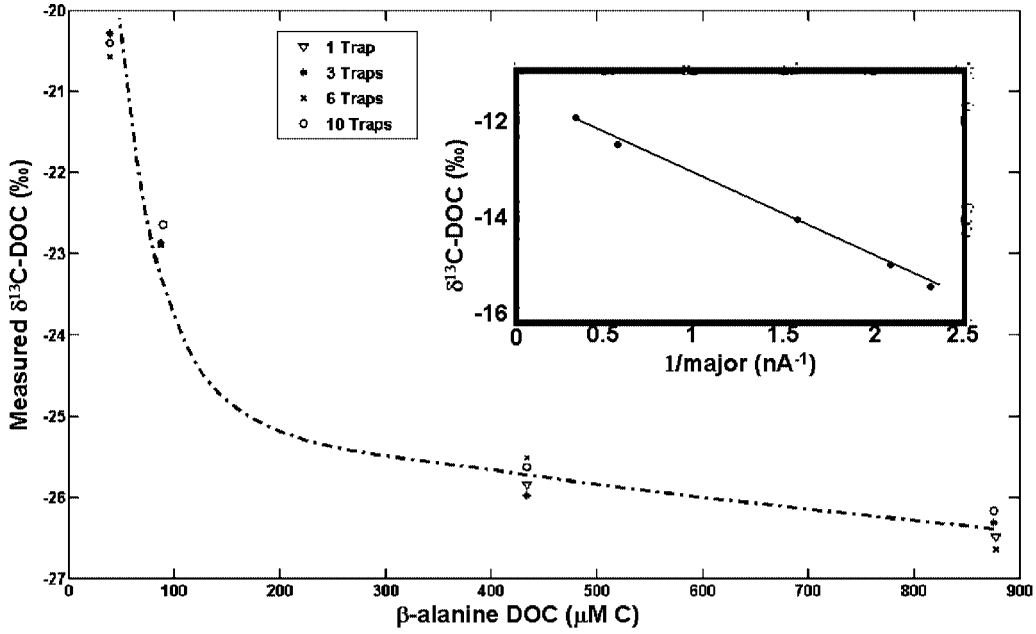

Figure 4. Concentration dependence of δ^{13}C-DOC of β-alanine standard solutions. Solutions of 39, 90, 433, and 875 μM C were trapped 3, 6, and 10 times each, with the two higher concentration solutions trapped once. Inset: Plot of IAEA-CH-6 δ^{13}C-DOC and the inverse of the IRMS signal intensity to determine blank contribution. See equation in text for details.

CO_2 that is trapped (Figure 4). This observation supports previous findings that blank contribution from HTC-based analyzers is constant[25,26] and could therefore be determined experimentally.

Our results revealed that extrapolation of the blank signal based on isotopic mass balance, as described,[27] from a total least-squares regression analysis of standard solutions is the most appropriate and convenient method to determine blank signal (eq 1):

$$\delta_m = \frac{(\delta_h - \delta_{bc})\eta_h}{\eta_m} + \delta_{bc} \quad (1)$$

Plotting the measured δ^{13}C (δ_m) as a function of the inverse of the measured sample intensity (η_m) gives the blank-corrected δ^{13}C (δ_{bc}) as the y-intercept (Inset Figure 4). Background δ^{13}C (δ_h) can be calculated from the slope once the intensity of the blank (η_h) is determined, either experimentally or through least-squares regression of NDIR and IRMS signal areas. Based on regular, three- or four-point calibrations with β-alanine and IAEA-CH-6 standard solutions, the blank in this system was calculated as 0.9−1.2 ± 0.1 nmol of C/min and δ^{13}C drifting between −15.51 and −17.78 ± 0.21‰ over a period of 3 months. Generally, the more depleted values would follow catalyst regeneration or periods when the TOC is used for NDIR quantitation and over the course of two or three days of regular use return to the more enriched background. These calculated results are validated by time-series traps of the background, which confirmed the magnitude (0.9 ± 0.1 nmol of C/min) and isotopic ratio (−15.39 ± 0.32 ‰, $n = 5$) of the blank. The variation noted above, particularly after catalyst regeneration, highlights the importance of daily measurement of background at the start and end of any run to detect minor drifts in the background. A typical analysis involving six traps is, on average, equivalent to ~20 nmol of C of background, or a signal intensity of ~0.11 nA, but has drifted over a span of five months between 0.15 and 0.04 nA. This system is capable of analyzing as little as 9.2 nmol of C for background, and six traps of deep marine samples is typically ~40 nmol of C (Figure S-1). We attribute the low IRMS detection limits to the fact that we bypass the EA combustion column. After determination of the blank isotopic ratio and signal intensity, the following isotope mass balance equation is applied to a measured sample (eq 2)

$$\delta_s = \frac{\delta_m \eta_m + \delta_h \eta_h}{\eta_m - \eta_h} \quad (2)$$

where δ_s is the stable isotope ratio of the analyte, with other parameters defined above. The procedure was first tested by deriving blank from IAEA-CH-6 calibration and applied to β-alanine standard solutions of 39, 90, 433, and 875 μM C and is as consistent with respect to accuracy and precision as by EA-IRMS (Table 2). Similar precision was obtained for IAEA-CH-6; however the isotope ratio was consistently depleted by ~1‰ compared to EA-IRMS analysis. This was attributed to a background contribution from the water used to dissolve the standard. Trapping ultrapure water injections showed the blank signal was consistently depleted compared to the instrumental background. The difference in peak height intensity of the ultrapure water was however not significant enough to differentiate quantitatively from instrumental background. The δ^{13}C of the contaminant in the ultrapure water was approximated to −29‰. Such a value is close to that of β-alanine, which may explain why no effect of this contaminant is evident in the more depleted standard. To confirm this hypothesis, a St. Lawrence River freshwater sample collected in June 2006 that has been measured for δ^{13}C-DOC by EA-IRMS[28] and HTC-IRMS (Table 2) was spiked with IAEA-CH-6 (at a 1:4.2 sucrose/natural sample ratio). Following blank correction and isotope mass balance calculation, the δ^{13}C-DOC of IAEA-CH-6 was comparable to the value obtained using the EA-IRMS. Since the typical δ^{13}C-DOC range of natural samples is −18 to −30‰,

Table 2. Accuracy of δ^{13}C-DOC Measurement for Standard Reference Compounds and Natural End-Members

				TOC-IRMS			EA-IRMS		
sample[a]	depth (m)	salinity[b] (psu)	[DOC] (μM)	δ^{13}C-DOC[c] (‰ vs PBD)	Std Dev	n	δ^{13}C-DOC[d] (‰ vs PBD)	Std Dev	n
β-alanine			45−1050	−26.59	0.29	13	−26.18	0.33	60
IAEA-CH-6[e]			106.6	−10.63	0.24	3	−10.62	0.19	29
IHSS SRFA			2188	−27.68	0.09	3	−27.6	0.12	na
DSS-CRM	2600	36.2	46.5	−21.37	0.33	3	−20.9		
St. Lawrence	40	0.1	424.9	−26.76	0.13	6	−26.8	0.2	2
Porewater[f]	2−3 cm	n/a	310.1	−22.84	0.16	3			

[a] See text for acronyms and sample details. Further details are found in Supporting Information. [b] Salinity determined in situ by conductivity detection. [c] After background correction of data (see text for details). [d] β-Alanine and IAEA-CH-6 are obtained from in-house EA-IRMS analysis. DSS-CRM and St. Lawrence are derived from the literature.[3,27] IHSS SRFA is the value reported by the IHSS, however sample number was not available. [e] From standard addition to St. Lawrence freshwater sample. [f] From the marine end of the St. Lawrence Estuary.

Table 3. Measured and Calculated Values for IHSS SRFA Standard Addition to a Marine End-Member[a]

standard addition concentration (μM C)	measured concentration (μM C)	measured δ^{13}C-DOC	calculated IHSS SRFA δ^{13}C-DOC	calculated deepwater δ^{13}C-DOC
0	62.8 ± 1.8[b]	−22.52 ± 0.11		
54.7	55.4 ± 2.0	−24.20 ± 0.38	−27.04 ± 0.27	−22.93 ± 0.69
109.4	117.5 ± 2.0	−25.38 ± 0.56	−27.70 ± 0.44	−22.28 ± 0.98
218.8	220.7 ± 0.5	−26.35 ± 0.17	−27.98± 0.45	−21.77 ± 0.48

[a] Four different solutions were prepared and analyzed at each standard addition level. [b] The value for the "0" addition is the natural concentration of the sample based on independent calibration and is subtracted from the total concentration to obtain the measured concentration of the spike.

mixtures of standards (2.2:1 and 6.9:1, β-alanine/IAEA-CH-6) were prepared at 125 and 68 μM C and, in both cases, match well with the calculated δ^{13}C-DOC (−21.46 vs −21.15‰ and −24.22 vs −24.04‰, respectively). Following the procedures outlined here results in a correlation coefficient of 0.993 between HTC-IRMS and other methods used to determine δ^{13}C-DOC (from published results or determined by EA-IRMS in our laboratory, data presented in Table 2). It should be noted that blank correction is not necessary for samples with DOC concentrations exceeding 600 μM C (e.g., wastewater, sediment porewaters, and others).

Accuracy of δ^{13}C-DOC Measurement. Because simple soluble organic compounds do not fully represent the structural and functional diversity found in natural DOC, and a consensus reference natural δ^{13}C-DOC sample is not available, accuracy was tested with the IHSS SRFA standard. The δ^{13}C-DOC of the SRFA stock solution was within ±0.1‰ of the value reported by IHSS (Table 2). Standard additions to a St. Lawrence Estuary marine end-member sample ([DOC] = 62.8 μM C, S = 34.5 psu) were carried out (Table 3, Figure 5 and Figure S-3). In these standard addition experiments, the δ^{13}C of the spike can be determined from the y-intercept of the linear relationship between the measured δ^{13}C-DOC versus the inverse of signal intensity,[12] and this experiment yielded an average δ^{13}C-DOC of the SRFA standard within ±0.2‰ the value given by the IHSS (Figure 5). In addition, mass balance calculations for both the natural DOC and SRFA standard agree with values determined experimentally for nonspiked sample and high concentration solutions (Table 3). Though our data agree with the reference isotope data given by IHSS, and IHSS humic substances were used to validate one WO-IRMS system,[12] we still feel an unprocessed high concentration DOC freshwater sample is needed to help anchor the analytical validation of δ^{13}C-DOC methods. This is particularly evident in light of the poor precision of the mass balance-derived δ^{13}C-DOC values compared to the experimental precision typically expected

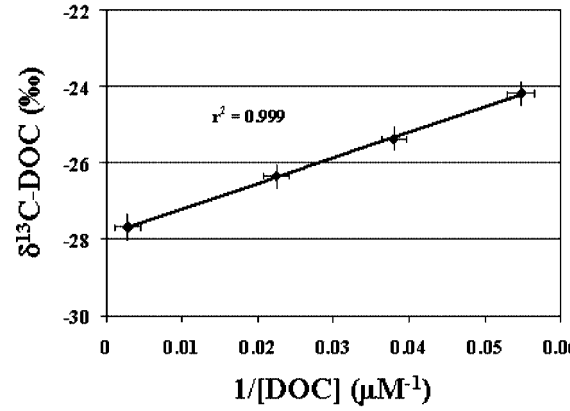

Figure 5. Standard addition of IHSS SRFA standard to deep Lower St. Lawrence Estuary. The intercept of the regression is the δ^{13}C of the spike compound[11] and is −27.8 ± 0.12‰, and r^2 = 0.99 (n = 12).

in both systems (Figure 5 of ref 12 and Table 3) and is likely due to solubility problems associated with these materials (Supporting Information).

Application to Natural Samples. The system was used to test extremes of natural samples to include high-concentration DOC freshwater (St. Lawrence River), low-concentration marine deepwater (DSS-CRM) (Table 2), and a stratified estuarine water column profile (Lower St. Lawrence Estuary) (Figure 6). After blank correction, the DSS-CRM and St. Lawrence River samples agree with literature data.[3,17,28] It should be noted that while the δ^{13}C-DOC value for DSS-CRM reported here is similar to what has been reported for off-line HTC-IRMS (−21.7 ± 0.29‰),[17] it is slightly more depleted compared to a more classic method (−20.9‰)[3] and significantly different from WO-IRMS (−19.5 ±

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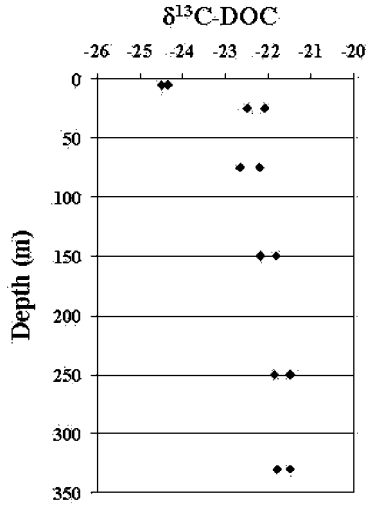

Figure 6. δ^{13}C-DOC depth profile for a station in the Lower St. Lawrence Estuary. Samples were measured in duplicate and never exceed more than 0.3‰ deviation. See Supporting Information for sample collection and processing details.

0.4‰),[11] highlighting the need for δ^{13}C-DOC intercalibration of selected natural samples. The estuarine water column profile shows conservative behavior of δ^{13}C-DOC, reflecting a salinity and temperature gradient in the upper 75 m of the water column, with no further variation with depth as well as demonstrating the precision typical of solid-state IRMS measurements (Figure 6). It should be noted the full profile was analyzed in one working day (12 sample injections, four standards). Finally, a marine sediment porewater sample was analyzed in triplicate and shows excellent precision for these high-DOC, complex matrix samples (Table 2).

The on-line HTC-IRMS system described here can withstand a large number (~300) of difficult sample injections (high salinity, high DOC, or both) before catalyst regeneration or fouling by salt deposition occurs and is at least as accurate and precise as other methods for δ^{13}C-DOC for a diverse range of sample types. It opens the possibility for large-scale routine δ^{13}C-DOC analysis of the most common natural sample types and should be of particular use toward a more complete understanding of DOC sources and cycling in complex environments with large DOC concentration gradients such as estuaries, coastal zones, and interstitial boundaries. If used in concert with the IRMS sensitivity increase reported in Osburn and St-Jean,[12] the sample throughput of HTC-IRMS could potentially increase as much as 2-fold. The current lack of certified standard materials can be side-stepped by standard addition of soluble reference standards to isotopically characterized natural samples. However, until the absence of certified reference samples representative of the structural and matrix complexity of natural DOC is properly addressed, assessing the isotopic accuracy of any δ^{13}C-DOC instrumentation will remain problematic. Ideally these references should be unprocessed natural samples and cover DOC freshwater and marine end-members; we suggest using DSS-CRM as the low-DOC high-salinity anchor. Future directions in HTC-IRMS system development should focus on further reducing the background signal of the TOC instrument to lessen the impact of blank correction on natural-abundance stable isotope analyses, which would also aid in reducing total analysis time and material consumption.

Dual isotope analysis, either isotopes of several atoms[29–31] or multiple isotopes of one element[1] has proven a highly effective method to decipher organic matter sources and cycling in a variety of materials (particulate matter, sediments, plants, etc.) from a number of ecosystems (oceanic, estuarine, lakes) but seldom carried on dissolved species because of difficulties outlined above. As measurement of δ^{15}N-DON has been demonstrated with high concentrations of standard compounds,[19] the feasibility of dual isotope carbon−nitrogen (δ^{13}C/δ^{15}N) analysis in natural dissolved organic matter samples should be explored using the HTC-IRMS approach.

ACKNOWLEDGMENT

This research was funded by the Natural Science and Engineering Research Council of Canada (NSERC), the Fonds Québécois de la recherche sur la nature et les technologies (FQRNT), and the Canadian Foundation for Innovation (CFI). R.J.P. extends thanks to Alexandre Ouellet and Alain Tessier for helpful suggestions through the development of this work, as well as Karine Lalonde and Jean-Francois Hélie for helpful discussion of this manuscript. The authors are also grateful to Edward D. Hudson for providing Deep Sargasso Sea Consensus Reference Material, and Ivan Markovsky for helpful advice on least-squares regression implementation in MATLAB. Two anonymous reviewers are also thanked for invaluable discussion and critique of the original manuscript. This is GEOTOP contribution 2008-019.

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review December 31, 2007. Accepted May 9, 2008.

AC702641Z

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