Automated quantitative and isotopic (13C) analysis of dissolved inorganic carbon and organic carbon in continuous-flow using a total organic carbon analyser

ARTICLE *in* RAPID COMMUNICATIONS IN MASS SPECTROMETRY · MARCH 2003

Impact Factor: 2.25 · DOI: 10.1002/rcm.926 · Source: PubMed

1 AUTHOR:

Gilles St-Jean
University of Ottawa
8 PUBLICATIONS **197** CITATIONS

SEE PROFILE

RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2003; **17**: 419–428
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/rcm.926

RCM

Automated quantitative and isotopic (^{13}C) analysis of dissolved inorganic carbon and dissolved organic carbon in continuous-flow using a total organic carbon analyser

Gilles St-Jean*

University of Ottawa, Department of Earth Sciences, G. G. Hatch Isotope Laboratories, Ottawa, Ontario, Canada

Received 2 October 2002; Revised 13 December 2002; Accepted 16 December 2002

A method for the automated ^{13}C analysis of dissolved inorganic and organic carbon species has been developed to operate on a continuous-flow isotope ratio mass spectrometer (CF-IRMS). For natural and anthropogenic carbon species, the ^{13}C stable isotope has proven to be an excellent environmental tracer. Analytical performance tests were carried out on various organic compounds from easily oxidisable (sugar) to difficult (humic acid). A set of natural samples was also analysed to confirm the flexibility of the system. Analytical precision (2σ) is typically $<0.20‰$ with sample reproducibility from 0.10–0.35‰ depending on reactivity of material. We believe this to be the first successful use of a total organic carbon (TOC) analyser for both dissolved inorganic and, specifically, dissolved organic species for ^{13}C stable isotope analysis in an automated CF-IRMS system. Routine analysis is achieved fairly quickly, is relatively simple with little or no sample manipulation, and will allow new and exciting studies for stable isotope research in both natural abundance and organic tracer studies not easily achieved before.

Dissolved carbon species, for aqueous systems, are classified in two categories: inorganic and organic carbon. All dissolved carbon sources are controlled by acid-base and redox reactions. Inorganic carbon is mainly controlled by acid-base reactions and exists in the form of dissolved CO_2, bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}), where species distribution is largely a function of pH. Organic carbon originates from chemical and physical changes in soil organic matter (SOM) that becomes soluble. Carbon transformations are linked through these acid-base and redox reactions, which are most often mediated by bacteria.[3] Analysis of dissolved organic carbon (DOC) species and dissolved inorganic carbon (DIC) forms the basis of carbon cycling studies. Schiff *et al.*[10] emphasise the importance of DOC in freshwater systems for the mobility of metals (e.g. chelate complexes) and other pollutants. For natural and anthropogenic carbon species, the ^{13}C stable isotope is an excellent environmental tracer.[4,11]

Most isotopic studies deal with well-established DIC methods to analyse ^{13}C.[3,14] Until now DOC analysis was difficult, time-consuming and often required considerable volumes of sample. This was true in low to sub-ppm levels of organic carbon particularly in freshwater systems. Another difficulty in obtaining reliable DOC values is that high molecular weight substances (humic acids) are insoluble below a pH of 2.[3,4] Acidification to remove the DIC from a sample would cause these base-soluble acids to precipitate. This in turn makes these precipitated compounds more difficult and resistant to oxidation.

Wassenaar *et al.*[12] used large volumes of water (10–120 L) to prepare DOC for geochemical and isotopic analysis including ^{14}C measurements. The samples were filtered using 0.45-μm inorganic filters, followed by liquid chromatography (LC) separation of hydrophobic (fulvic *and* humic acids) fractions on a XAD-8 column. The humic acids were precipitated by bringing the base effluent to a pH of 2 and leaving to settle for 24 h. The fulvic acids were then freeze-dried creating a water-soluble product for analysis. Rao and Killey[9] developed a method for quantitatively separating the DIC and DOC fractions for ^{14}C studies. In this case a sample of approximately 1.5 L was used, and the DIC was separated by acidification and the DOC by chemical oxidation so that the evolved gases were eventually converted to $BaCO_3$ and quantitatively weighed. Hood *et al.*[15] used both XAD-8 and the XAD-4 LC columns (for the hydrophilic fraction, nonhumic, see Aiken *et al.*[16]) to separate components of the DOC, but in this case for dissolved organic nitrogen (DON) analysis. Le Clercq *et al.*[5] used supercritical oxidation of marine DOC to determine ^{13}C ratios. The technique involves having the sample in a ceramic tube and bringing it to a supercritical state in a 650°C oven at 350 bars using an HPLC pump with oxygen as the oxidant and copper as the catalyst. The evolved CO_2 is cryogenically trapped in a vacuum vessel and analysed on a dual-inlet IRMS with precisions of about 1‰ for $\delta^{13}C_{PDB}$ and 3% for DOC concentration.

A method for the automated ^{13}C analysis of dissolved inorganic and organic carbon species has been developed to operate on a continuous-flow isotope ratio mass

*Correspondence to: G. St-Jean, University of Ottawa, Department of Earth Sciences, G. G. Hatch Isotope Laboratories, Ottawa, Ontario, Canada.
E-mail: gstjean@science.uottawa.ca

spectrometer (CF-IRMS). Preliminary work on this was first presented by the author in 2000 at the Canadian Continuous-Flow IRMS Workshop in Montréal, Canada, and was first reported at the AIG-IV conference in 2001.[18] It allows for the sequential analysis of up to 53 liquid samples containing carbon contents of 0.2–35 ppmC for DIC and/or DOC. Larger concentrations (up to 10000 ppmC) are analysed using a microsampling syringe giving an effective carbon signal in the 2–60 ppmC range, thereby matching the linear dynamic range of the mass spectrometer. Sampling is performed using either a sampling loop (low concentration, automatic mode) or a syringe via septa injection (high concentration, single mode). A secondary method is to dilute the original samples to match the analytical concentration within the IRMS and TOC analyser range using ultrahigh purity deionised water and high precision pipetters if quantitative concentrations are needed. A third way of analysing larger samples is to use the automated helium dilutor built into the ConFlo® III IRMS interface.

The method of analysis for ^{13}C isotope content in DIC is well known. Typically, acidification (H_3PO_4) is used to react with the DIC and the evolved CO_2 is either cryogenically trapped for later dual-inlet analysis, or is sampled by an automated GC-type of device (i.e. GasBench II, Finnegan MAT) and brought to an open split for continuous-flow isotope ratio mass spectrometric analysis. The DOC has always been a more difficult sample to analyse for isotopes due to the lack of available instrumentation to interface with the IRMS, presence of compounds difficult to convert to CO_2, and usually low organic carbon concentrations in natural waters.

Acronyms and definitions for the different types of inorganic and organic carbon found in the literature can be at times perplexing. Table 1 lists the most common definitions which may vary depending on the field of study (Oceanography, Biology, Toxicology, Water Quality Controllers, etc.).

Methods of quantitative DIC/DOC analysis

The most common methods for quantitative analysis of DIC and DOC are achieved using what are referred to as TOC analysers. Each type uses either ultrahigh purity (UHP) N_2 or UHP air as the carrier or carrier oxidiser. There are generally two types of analysers each with their pros and cons.

Table 1. Common definitions for carbon species in aqueous systems[5–7]

TC	Total Carbon. TC is all the carbon in an aqueous sample including inorganic, organic and volatile carbon species. TC = TIC + TOC.
TIC	Total Inorganic Carbon. TIC is all the carbon in a sample that is converted into CO_2 after sample acidification. TIC includes dissolved CO_2 as well as bicarbonate and carbonate species ($CO_{2(aq)}$, HCO_3^-, CO_3^{2-}) as well as particulate matter.
DIC	Dissolved Inorganic Carbon (0.45-m filtered sample). DIC = $CO_{2(aq)} + H_2CO_3 + HCO_3 + CO_3^{2-}$; where the relative concentration of each component is a function of pH.[1]
TOC	Total Organic Carbon (organic compounds converted into CO_2 by oxidation). Also known as total oxidisable carbon. TOC = POC + NPOC. TOC is all the carbon in organic compounds that is converted into CO_2 by oxidation after the inorganic carbon (TIC) has been removed. Although TOC in water samples should ideally include carbon from volatile substances, most laboratories report TOC analyses of samples where volatiles have been previously removed. The widely accepted methods involving persulphate oxidation call for acidification and purging to remove inorganic carbon before oxidation of organics. Purging can also remove volatile organics before oxidation, although the results are still generally accepted as TOC. Volatiles can be included in TOC by separately measuring TC and TIC and calculating TOC by difference. NOTE: For marine chemists the definition is slightly different where TOC = SOC + DOC
DOC	Dissolved Organic Carbon (0.45-m filtered sample). DOC is organic carbon determined by the analysis of aqueous samples that have been filtered through 0.45-m filters.[1] This definition may vary, depending on who you talk to, mainly due to using different filter sizes; e.g. in some instances 0.20-m filters are used.
POC or VOC	Definition 1: Purgeable Organic Carbon or Volatile Organic Carbon. Organic carbon purged from solution by a stream of gas under a specific non-standardised set of purging conditions. Definition 2: Particulate Organic Carbon; see POM.
NPOC	Nonpurgeable Organic Carbon. Organic carbon that remains in solution after a sample has been purged by a gas stream under a specific set of purging conditions. NPOC is often reported as TOC due to popular methods requiring acidification and purging of TIC prior to oxidation of organics. This substitution is valid for samples containing negligible volatile or purgeable organic compounds.
SOC	Suspended Organic Carbon. Organic carbon determined by the analysis of particles captured by the filtration of aqueous samples through a 0.45-μm filter. SOC is sometimes called particulate organic carbon. It is reported as total mass of carbon. Sedimentary Organic Carbon.[3] Undissolved organic carbon usually found in an aquifer.
PAH	Polyaromatic Hydrocarbons or Polycyclic Aromatic Hydrocarbons are a group of over 100 different chemicals that are formed during the incomplete burning of coal, oil and gas or garbage. Examples of PAHs are anthracene, phenanthrene, fluoranthene, chrysene and pyrene.
NOM	Natural Organic Matter: occurs in surface as well as groundwater and consists of both humic (i.e., humic and fulvic acids) and non-humic components. NOM = COM + DOM + POM.
COM	Colloidal Organic Matter
DOM	Dissolved Organic Matter; see DOC. Other forms of DOM identifications are: coloured DOM (CDOM) and is mainly produced by bacterial activity. High molecular weight DOM (HMW-DOM). Low molecular weight DOM (LMW-DOM).
POM	Particulate Organic Matter; see SOC. POM is created by both biotic (bacterial uptake) and abiotic (e.g. coagulation of colloidal particles) processes.
FPOM	Fine Particulate Organic Matter; see POM
SOM	Soil Organic Matter. Organic matter available for physical and chemical processes (e.g. bacterial decomposition).
ppmC	Parts-per-Million Carbon. Mass units of solute (carbon) per million sample mass units of solution. In aqueous samples this is generally the same as mgC/L or μg/mL.
ppbC	Parts-per-Billion Carbon. Mass units of solute (carbon) per billion sample mass units of solution. In aqueous samples this is generally the same as μgC/L or ngC/mL

Low- (680°C) and high-temperature combustion (800–950°C)[6,7]

These classic methods combust samples to CO_2 in an O_2 atmosphere at high temperature with a primary catalyst of Pt on alumina beads, Pt-coated quartz wool or crushed quartz. The choice of catalyst is based on combustion temperature and application. The evolving gas is usually measured by a nondispersive infrared (NDIR) detector to obtain a TC value. A second separate TIC measurement is achieved by acid reaction which is then subtracted from the TC to give the TOC. Samples are typically introduced into the instrument by syringe injection.

The advantages of these combustion methods include better oxidation of refractory OC such as humic and fulvic acids, high levels of TOC are easily analysed, rapid analysis (2–3 min), and low sample volume (hundreds of μL, syringe injection). The disadvantages include a limitation to medium to high levels due to small sample sizes, TOC is determined by difference (TOC = TC−TIC) and cannot be extended for isotope work, system blanks are caused by the catalyst, and catalyst poisoning.

Wet oxidation method

This method converts organic compounds to CO_2 by chemical and/or ultraviolet (UV) decomposition directly in the water sample. There are four main methods for wet oxidation of DOC samples.[6]

UV only

UV light converts organics in a sample into CO_2. The level of organics is detected by a change in conductivity using a thermal conductivity detector (TCD).

UV/persulphate

Sample is added to persulphate (Na, K, or ammonium) in a UV-irradiated chamber where the organic carbon is converted into CO_2 and is detected by an NDIR or a TCD.

UV/TiO_2

Similar to UV/persulphate but TiO_2 is used as the catalyst.

Heated persulphate (e.g. $Na_2S_2O_8$) or 100°C/persulphate

Persulphate is added to the sample in a chamber heated to 95–100°C, converted to CO_2 and detected by an NDIR. This method is the one chosen in our evaluation of the analytical instrument for isotopic analysis. The advantages of this method are: low-level TOC determination is possible due to larger sample sizes (up to 25 mL) thus increasing sensitivity to lower ppb levels; automatic carousel analysis for large sample throughput via sampling loops; each sample receives an independent aliquot of reagent thereby eliminating the common catalyst poisoning of the combustion method; depending on specific method and detector types, samples containing salts and particulates may be easily analysed (extra filters are needed for halogen removal in both quantitative and IRMS analysis in high salinity samples); TOC is determined directly, not by difference; isotope work on DOC is possible. The disadvantages of the method are: slow analysis times (6–15 min); high levels of TOC may be difficult to analyse directly, but can be accomplished by high precision dilution.

EXPERIMENTAL

Pictures of the equipment used can be found at the website of the University of Ottawa's G.G. Hatch Isotope laboratories[19] by following the link to ''Instruments and Equipment''. The set-up consists of a Finnegan MAT (Bremen, Germany) Delta Plus IRMS with a ConFlo® III continuous-flow interface; a CE Instrument (Milan, Italy) model 1110 elemental analyser to calibrate the standards (EA-IRMS); a modified OI Analytical (College Station, TX, USA) model 1010 wet oxidation TOC analyser with a 53 sample autosampler model 1051 with septum piercing capability and magnetic stirring. Vials used on the TOC analyser are TraceClean™ pre-cleaned 40-mL, amber borosilicate, with 0.125″ septa liner, also known as ''environmental sample containers'' (VWR cat# 15900-024). Deionised water is provided by a Barnstead NANOpure 4-cartridges water filtration unit providing high purity water at >17 MegOhms/cm.

For the TOC analyser system to work in continuous flow certain criteria have to be met.

1. The instrument must be able to use He as carrier gas instead of the usual N_2 without the NDIR being damaged due to a He-porous window.
2. Sample carrier flow rate should be similar to that used in either a gas chromatograph or an elemental analyser in order to utilise existing IRMS interfaces and control methods. In this case the EA flows are more appropriate.
3. Sample trigger of the TOC analyser *must* be controlled by the IRMS software to eliminate timing problems.
4. There is a bonus if the TOC analyser software can perform the quantitative analysis at the same time.

TOC analyser hardware and software (WinTOC-1010) modifications by OI Analytical now allow mass spectrometer control of the sequence, thereby eliminating timing problems between two independent software packages. In a Windows™ environment both the IRMS and the TOC analyser's control software can run on the same computer. The analytical mode for the IRMS was set using the well-known elemental analyser IRMS[2] (EA-IRMS) for method development and sequence preparations. A scrubber-conditioning interface (patent pending) was designed to be placed between the TOC analyser and the ConFlo interface (Fig. 1). The same sample determination of both DIC and DOC is performed by wet oxidation using the model 1010. The first step is sample acidification (5% H_3PO_4) to convert DIC into CO_2. Since the reaction takes place in a closed vessel (Fig. 1), the method can be programmed to react for any length of time. This is of particular importance in the DOC reaction where time and reagent quantity are more critical for organic breakdown. The gases are then purged from the sample, followed by moisture removal via Nafion™ tubing and indicating desiccant. Carbon dioxide is then selectively measured by a NDIR detector calibrated to measure quantitative CO_2 that is proportional to the DIC in the sample. After the DIC has been completely removed from the sample, the persulphate reagent is added. The persulphate oxidises, at 100°C, the organic carbon in the sample to form CO_2, which is later purged from the sample and

RCM

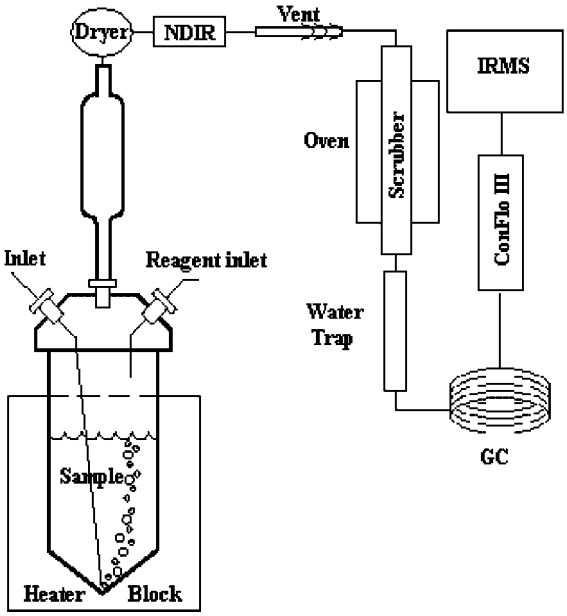

Figure 1. OI Analytical TOC analyser model 1010 reaction chamber with attached interface.

measured at the NDIR detector. For isotope work, the evolving CO_2 is tapped at the vent and sent through additional water and halogen traps. The extra water trap consists of magnesium persulphate, and the halogen traps contain electrolytic copper with and without added silvered cobaltic-cobaltous catalyst at 750°C, similar to that of a typical NC elemental analyser configuration. The scrubbed CO_2 is then focused using a packed NCS GC column and sent to the ConFlo III IRMS interface followed by isotopic analysis. The model 1010 has a total gas consumption of 300 mL/min. The sample carrier gas is set to 110 ± 10 mL/min and the balance of He is used to sparge the reagents, reactor rinsing water and to keep the Nafion® moisture removal trap dry. At such high consumption it would be costly to operate the TOC analyser on He alone. A switching valve is used to choose between N_2 and He as the carrier gas. Analytical precision for the model 1010 on quantitative TOC analysis is 2 ppbC or 2% (whichever is higher).

Preliminary DIC/DOC isotopic tests

Data are reported using the delta (δ) notation defined as:

$$\delta^{13}C_{sample} = (R_s/R_{st} - 1) \times 1000$$

where R_s is the ratio of $^{13}C/^{12}C$ in the sample and R_{st} is either the ratio of the international standard (PDB) or an internal reference gas (relative delta). Analytical precision (2σ) is typically <0.20‰ with sample reproducibility from 0.10–0.35‰ depending on reactivity of the material. An initial investigation was performed using the products in Table 2 with and without a GC column between the TOC analyser and the IRMS ConFlo interface. Note that the preliminary data are *relative* to the reference gas and not calibrated to a scale.

By adding the separation column, the standard deviation is improved by a factor of 7 for the DIC. The effect on the DOC is not as significant due to the initial purging of the DIC out of the reaction vessel bringing with it most of the other dissolved gases. Yet, with the DOC less affected by the interfering masses, the data after the GC column are still 2‰ higher on the three first organic compounds. This indicates that, even after sparging the DIC along with other dissolved gases, we still need to chromatographically separate the evolving CO_2 and somehow clean out the other contaminants (halogens, VOC etc.).

The IRMS traces (Fig. 2) for the $^{45}/_{44}$ ratios and m/z 44 peaks indicate that samples without the GC column show definite signs of contamination. We observe on the ''With GC Column'' trace amplified noise due to higher sensitivity of the ratio analysis as opposed to straight beam intensity measurement. Figure 3 shows the TOC analyser software simultaneously acquiring quantitative information. This is most evident for the DIC, but the DOC $^{45}/_{44}$ traces also show some contamination and is most likely due to two factors, namely, interference caused by trace gases (halogens,[17] S species, moisture) evolving from the sample and interfering with CO_2 ionisation in the mass spectrometer source, and dissolved N_2 levels so high that backscattering occurs in the flight tube. The $^{45}/_{44}$ and $^{46}/_{44}$ ratios are particularly affected due to the 100-to 300-fold amplifications relative to m/z 44 required for m/z 45 and 46, respectively.

Scrubber-conditioning interface

This part of the system was designed to remove (scrub) contaminants and to chromatographically split any components that could not be separated or removed physically or chemically. This conditions the signal from the effluent of the TOC analyser to mimic the output from an EA, thus allowing the entire sample to be measured by the IRMS. The difficulty is in part due to the fact that the model 1010 works at high carrier flow (110 ± 10 mL/min), for the NDIR to work properly, but at low pressure. Typically, the internal operating pressures are between 3.5 (0.5) and 69 kPa (10 psi). The EA works at higher carrier pressures (150 kPa; 22 psi) to push through the combustion-reduction, water and/or CO_2 traps and the GC column, maintaining a He carrier flow between 80–140 mL/min. At too high a carrier gas pressure, the model 1010 gives an overpressure error, and, potentially the NDIR detector could be damaged.

Table 2. Results of test samples with and without a GC column between the TOC analyser and the IRMS interface

	Without GC column		With GC column	
Sample	Rel. Delta (‰)	Std dev (95%)	Rel. Delta (‰)	Std dev (95%)
Raw sugar (10 and 15 ppm)	30.81	0.29	32.81	0.15
Sucrose (20 ppm)	31.87	0.25	33.59	—
Atropine	13.46	0.21	15.54	—
Ammonium bicarbonate	42.67	1.51	40.71	0.21

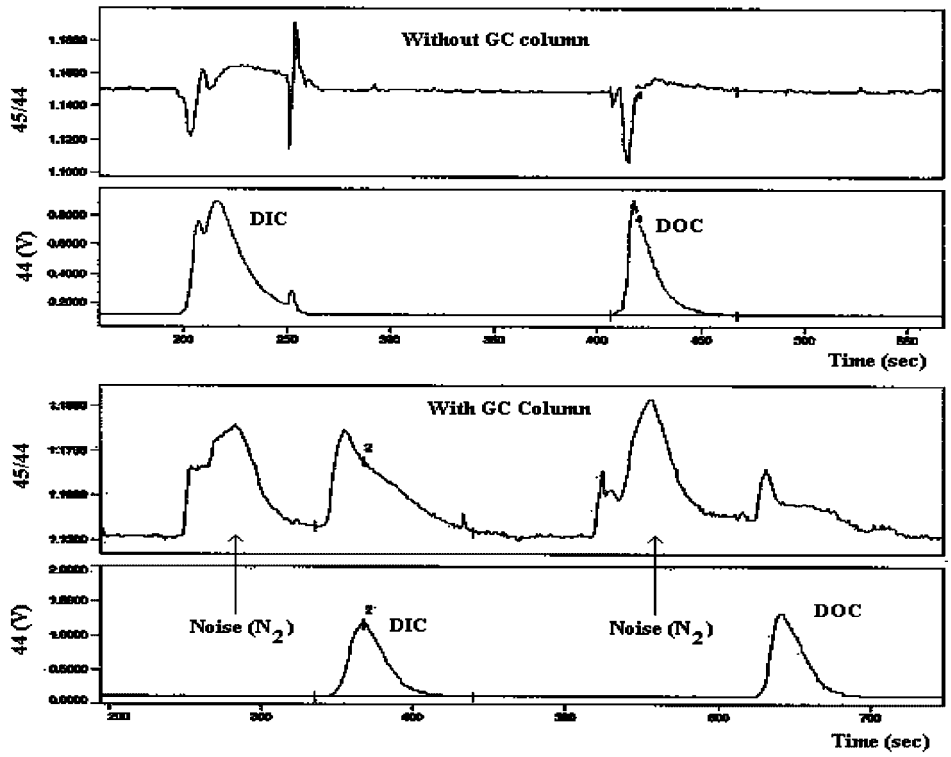

Figure 2. Comparison traces of the evolved CO_2 from the TOC analyser with and without the NCS GC separation column. Note the noise on the *m/z* 44 trace before and after the GC column was added.

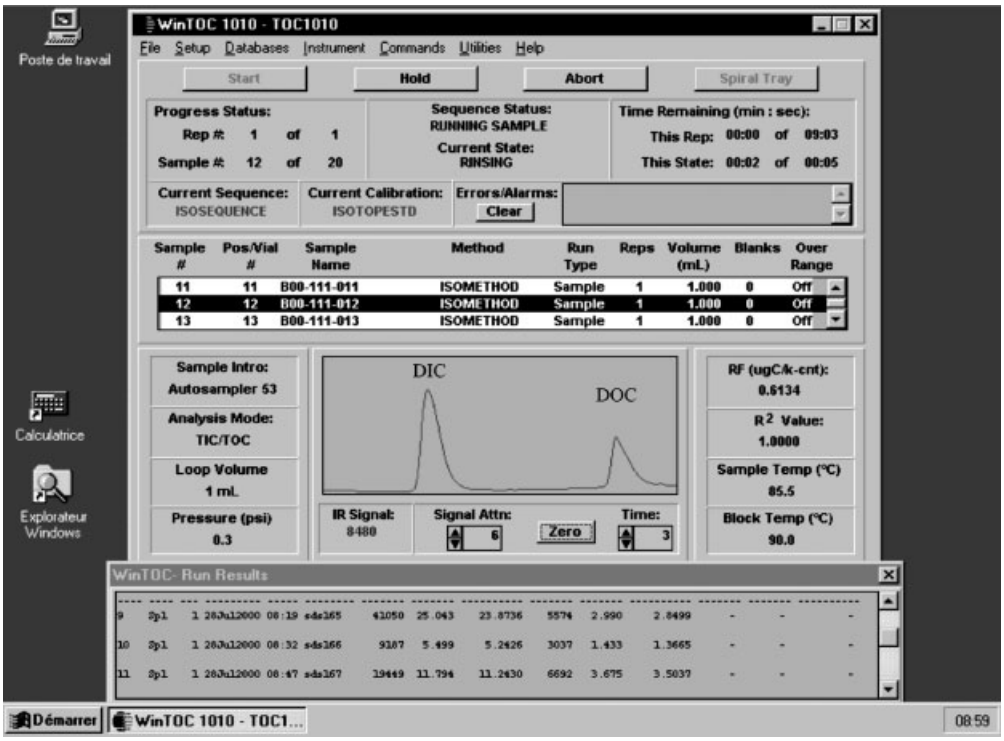

Figure 3. TOC analyser software (WinTOC™ 4.1; OI Analytical).

Calibration method

A set of organic compounds was selected from easily oxidisable (sucrose) to difficult refractory materials (humic acid). The samples, some from the EA quantitative standards, were analysed straight out of the bottles for isotopic composition on the EA-IRMS, using IAEA and NBS isotopic reference materials to calibrate and normalise the data. The final set of test samples was chosen based on both chemical composition (6–17 carbon-atom compounds plus the heterogeneous mixture known as humic acid; the products also had

RCM

Table 3. Organic compounds selected for the IRMS evaluation. Std Dev. on $\delta^{13}C_{PDB}$ EA-IRMS analysis is typically <0.20‰. Sample reproducibility is 0.11–0.33‰

Product	Molecular formula	$\delta^{13}C_{PDB}$ of powder (‰)	$\delta^{13}C_{PDB}$ (liquid) (‰)
Atropine-2	$C_{17}H_{23}NO_3$	−28.39	−28.13
Citric acid	$C_6H_8O_7\dot{c}H_2O$	−15.72	−16.39
Humic acid, sodium salt, tech (Heterogeneous refractory compound)	—	−25.59	−25.70
Nicotinamide-5	$C_6H_6N_2O$	−32.71	−32.75
Potassium biphthalate	$C_8H_5KO_4$	−23.57	−24.48
Sucrose-A	$C_{12}H_{22}O_{11}$	−10.20	−11.55
Sulphanilamide-6	$C_6H_8N_2O_2S$	−29.51	−29.47
Urea-7	CH_4N_2O	−40.37	−39.89
Low cal sugar	—	−11.70	−15.47
Raw sugar	$C_{12}H_{22}O_{11}$	−10.61	−11.86

to be *fully* soluble in water at room temperature thereby eliminating samples such as BBOT and cyclohexanone), and on the isotopic composition spread in $\delta^{13}C_{PDB}$ (Table 3).

Initial solutions of 1000 ppmC of each product were prepared, giving an effective carbon content of 10 µgC/10 µL. All borosilicate glassware used for the experiments was cleaned with high-purity deionised water followed by burning off of organics in the glassblower's annealing oven. Multiple aliquots (10 µL) of each solution were prepared in individual tin capsules for EA-IRMS analysis. Standard deviations of $\delta^{13}C_{PDB}$ for analysis on the EA-IRMS on all products were typically better then 0.20‰. Note that the values for the solid sugars are different from those for the samples recrystallised from solution by at least 1.25‰. It is suspected that the sugars may have been affected isotopically by the pre-weight drying process following the United States Pharmacopeia (USP) Standards preparation method of drying at 110°C. This isotopic shift was not observed for straight sugar samples. Further study of this effect is being conducted. Surprisingly, all other products correspond fairly well to the initial solid powder, including the humic acid compound.

It is well known that isotopic homogeneity in a crystallised solid is often difficult to achieve. To counter this possible effect in the powders, each solution was individually calibrated for use on the TOC analyser.

Instrument performance

Analytical conditions for the TOC-IRMS are shown in Table 4. The oxidant reaction time is generally constant except for timing experiments performed on the humic acid. In all isotopic continuous flow systems, it is necessary to know the mass spectrometer's linearity before any measurement is performed, unlike for dual-inlet analysis where the reference and sample gases can be adjusted to the same pressure (m/z 44 intensity) via adjustable bellows. Continuous flow does not allow that luxury since the sample is a single pulse and the ratios are calculated from the scanned areas under the curves for m/z 44, 45 and 46. Linearity, in a nutshell, is the capability of the electron impact (EI) source of the IRMS to ionise a gas (e.g. CO_2) at different pressures and give within certain specifications (≤0.02‰/nA) the same isotopic ratios (e.g. $^{45}/_{44}$, $^{46}/_{44}$).

Sample preparation

When analysing solid organic materials for nitrogen, carbon or sulphur isotopes in an EA-IRMS, the NCS content (%) of the sample is needed first. Otherwise blind preparation takes place in sample weighing, and the mass spectrometer's dynamic analytical range may be missed and potentially the sample could be wasted due to wrongful assumptions made when preparing it. Samples may have to be first analysed using a quantitative EA to obtain the NCS information (e.g. soil, mud, etc.). The same rules apply here for a TOC-IRMS. Samples are first analysed for quantitative DIC and DOC on the TOC analyser and then properly prepared for isotopic measurement. If one of the two species types, DIC or DOC, are out of range, then a separate sample is prepared according to a precise dilution technique. A combination of

Table 4. TOC analyser analytical conditions

Delta^{Plus} IRMS		TOC Analyser	
Trap (µA)	800	***Acid*** (5% H_3PO_4)	300 µL
		Reaction time	02:00 min
Linearity (‰/nA) from 1–7 V or 3.3–23.3 nA	0.014	Detection time	01:00 min
		Reaction temperature	90°C
Sensitivity (mol/ion)	1250	***Oxidant*** (200 g/L $Na_2S_2O_8$)	800 µL
		Reaction time	03:00 min
ConFlo Interface	II and III	(except where noted)	
		Detection time	01:30 min
		Reaction temperature	97°C
Differential pump	yes	***Rinse*** (Nanopure water)	25 mL
		Rinses per sample	2
		Rinse temperature	85°C
		Standby temperature	85°C
		Sample loop (calibrated)	1.05 mL

Table 5. Results of DOC analysis on sucrose and humic acid. Statistics for humic acid include various reaction times

	Sucrose-B	Humic Acid Sodium salt, tech
TOC Reaction time (min)	3.5	3.5–10.5
Mass/Aliquot (μgC)	20–50	15–40
TOC Conc. (ppmC)	9.274	7.853
IRMS Conc. (ppmC)	9.281	7.847
IRMS Mass 44 (V)	1.2–3.0	1.2–2.5
IRMS Mass 44 (nA)	4.0–10.0	4.0–8.3
$\delta^{13}C_{PDB}$ (‰)	−11.06	−25.80
Std dev (‰)	0.20 (n = 10)	0.35 (n = 12)
95% Confidence (‰)	0.14	0.25

burette and high precision micropipetters provides a quick way of preparing the samples in TraceClean™ 40-mL sample vials for the TOC analyser's autosampler.

Experimental performance

Two experiments were conducted to investigate the performance of the TOC-IRMS. The first examined the reproducibility of an easy (sucrose) and a difficult (humic acid) material for oxidation (Table 5). The second analysed a few real samples from various sources (Table 6).

Table 5 describes two materials prepared for reproducibility tests at a fixed concentration of about 10 ppmC, as well as sample linearity at varying sample sizes. All aliquots were analysed using a method for simultaneous DIC and DOC on the TOC analyser.

The sucrose-B is from a source different from that for the standard sucrose-A (see Table 3), and the humic acid is the same product. The humic acid (Aldrich, cat# H1,675-2) is similar to that used by Rao and Killey.[9] The mass of carbon (μgC) indicates the sample size range used. In the case of sucrose-B this was 20–50 μgC indicating 2–5 mL aliquots per analysis. The mass spectrometer software (Isodat™) has the capability to calculate quantitative information that is proportional to the *m/z* 44 peak area (Vs). The software uses a K-factor type of regression where the default units (hardcoded) are 'weight percent' (%) and the sample size is in 'milligrams'. Since the K-factor equation is independent of units, providing we are consistent the 'weight percent' can be replaced with ppmC, and the weight (mg) can be replaced with volume (mL or μL). This may be useful should the NDIR

Table 6. Samples for DIC and DOC analysis taken from different natural water sources. o.r. = out of range for IRMS; n.a. = non applicable due to rough dilution; DIW = deionised water

	DIC			DOC		
Sample	Conc. (ppmC)	Mass (gC)	$\delta^{13}C_{PDB}$ (‰)	Conc. (ppmC)	Mass (μgC)	$\delta^{13}C_{PDB}$ (‰)
Tap water (4 mL) (Aylmer, Québec)	3.41	14.30	−12.09	3.77	15.80	−25.27
Tap water from lab (4 mL) (Ottawa, Ontario)	3.47	14.56	−12.06	3.69	15.48	−25.14
Rideau canal water (3 mL) (Ottawa, Ontario)	8.21	25.85	−8.85	12.97	40.82	−20.23
Suzanne's well water (1 mL) (Fall 2001, Aylmer, Québec)	55.97	58.71	−15.00	3.04	3.18	−23.88
Suzanne's well water *before* water softener.						
—1-mL aliquot for DIC	55.92	58.66	−15.61	0.73	0.76	−23.37
—5-mL aliquot for DOC	60.64	381.69	o.r.	1.91	12.02	−23.41
(Spring 2002, Aylmer, Québec).						
Suzanne's well water *after* water softener.						
—1-mL aliquot for DIC	53.88	56.52	−15.71	0.87	0.91	−23.73
—5-mL aliquot for DOC	57.79	363.75	o.r.	2.17	13.66	−23.40
(Spring 2002, Aylmer, Québec).						
B01-189-10 (Ceiling dripping site-1; (06/10/2001)						
—1-mL aliquot used for DIC and DOC	49.793	52.233	−11.70	6.562	6.883	−26.41
(Moonmilk study; Caverne de l'Ours, Perkins, Québec)						
B01-189-25 (Ceiling dripping site-1; 2001-12-08)						
—2-mL aliquot for DIC and DOC	36.19	75.93	−13.28	4.11	8.63	−26.42
—2-mL aliquot with approx. 75% dilution in DIW	n.a.	18.57	−12.88	n.a.	1.29	−26.15
(Moonmilk study; Caverne de l'Ours, Perkins, Québec)						
B01-189-27 (Ceiling dripping site-4; 2002-05-02)						
—2-mL aliquot for DIC and DOC	28.60	60.01	−10.77	3.30	6.93	−25.77
—2-mL aliquot with approx. 50% dilution in DIW	n.a.	14.98	−10.38	n.a.	3.11	25.31
(Moonmilk study; Caverne de l'Ours, Perkins, Québec)						
B01-189-24 (Cave spring, site-4; 2001-12-08)						
—4-mL aliquot for DIC and DOC	15.48	64.97	−7.72	3.92	16.46	−26.84
—4-mL aliquot with approx. 50% dilution in DIW	n.a.	32.91	−7.54	n.a.	7.97	−26.33
(Moonmilk study; Caverne de l'Ours, Perkins, Québec)						
B01-189-28 (Cave spring, site-4; 2002-05-02)						
—4-mL aliquot for DIC and DOC	25.55	107.21	o.r.	4.02	16.87	−26.12
—4-mL aliquot with approx. 75% dilution in DIW	n.a.	24.99	−8.02	n.a.	3.15	−25.96
(Moonmilk study; Caverne de l'Ours, Perkins, Québec)						

on the TOC analyser fail. When compared with the average simultaneous TOC analyser concentrations, the concentrations derived using the IRMS are quite good for the different sampling sizes used, in this case better than 1 ppmC. The IRMS m/z 44 signal is indicated in both volts (V) along with nanoampres (nA), for comparison only, and is an indicator of the sampling range used. Reproducibility of the $\delta^{13}C_{PDB}$ values is excellent for such small quantities of sample.

Different aliquots of humic acid, a complex mixture of refractory compounds, were reacted with the oxidant for varying amounts of time. Out of the 12 aliquots analysed, four were reacted in increments of 2 min, and all results are included in the calculation of the average and the standard deviation. The SD of the four taken as a group (0.28‰) fall well within the SD of the entire group (0.35‰). The average precision for isotopic analysis is in most cases $\leq$ 0.20‰, which is comparable to present-day EA-IRMS performance.

Samples were taken from various natural water sources in the Ottawa, Ontario region, to check the instrument performance (Table 6). Quantitative calibrations (ppmC) were calculated using 5, 10 and 20 ppmC standards.

Tap-water samples were taken on the same day from a home in Gatineau (Aylmer sector), Québec, and from our laboratory in Ottawa, Ontario. The water treatment plants of the two municipalities get their water from the Ottawa River within 7 km of each other. The data show the same quantitative and isotopic values for both DIC and DOC. Government guidelines for TC are around 6 ppmC. Further investigation has shown that both municipalities have similar treatment plants, with some differences in final water handling. Water disinfection in Aylmer uses only gaseous chlorine, whereas the Ottawa system uses monochloramines and adds fluoride to the water by means of $HFSi_6$, neither of which adds or creates carbon compounds that are detectable by quantitative or isotopic shifts. This result may have some interesting applications in water quality monitoring, where ^{13}C isotopes of DIC and DOC could be used to trace bacterial or anthropogenic contamination in communities.

The Rideau Canal is an artificial construction of locks linking the Ottawa River to a system of lakes and rivers that spans from the city of Kingston on Lake Ontario to the city of Ottawa. It was built over 169 years ago through carbonaceous rocks along with cement walls and sewers. The area where this sample was taken tends to be shallow and partially stagnant, where decomposition of organic matter is high (strong swampy smell). During intense rain events, the surrounding area's surface waters flow into the canal. High algal growth is evident in this area of the canal, and may be due to heavy use of fertilisers, pesticides and herbicides on lawns and agricultural lands. Upstream runoff from the agricultural area surrounding the Rideau Canal Waterway system is a major contributor of organic materials to these waters. This is apparent from the DOC concentration at 13 ppmC compared with that for the other natural water test samples. The main agricultural product from Ottawa to Kingston is corn for human and animal consumption; the unused portions of the plant are often mulched and returned to the soil as fertiliser. This could explain the 5‰ difference in the $\delta^{13}C_{PDB}$ of the canal DOC (−20‰) from the other local values (−24 to −26‰). Corn is a C_4 plant with an average isotopic composition around −12.5‰. Since most of the plants in this area are of the C_3 species with an average $\delta^{13}C_{PDB}$ around −27‰, the contribution from corn matter decomposition would tend to shift the DOC towards the positive, in this case from the local average of −25‰ to the canal value of −20‰. The DIC values of all samples are typical of a carbonate terrain where calcite dissolution by carbonic acid from soil CO_2 occurs in a partially open system, achieving almost a 50:50 balance between the $\delta^{13}C_{PDB}$ DOC and DIC. This reaction can be written as indicated by Clark and Fritz:[3]

$$CO_{2(g)} + H_2O + CaCO_3 \rightarrow Ca^{2+} + 2HCO_3^-$$

Suzanne's well water is from a daycare home in Aylmer, Québec, where a test was performed to see whether differences could be detected between water directly out of the well and water running through the water softener. Hard water is high in dissolved minerals, usually Ca and Mg, causing limescale build up in the water pipes and poor soap/detergent performance. Water softeners contain Na salts that replace Ca and Mg therefore softening the water. The data show clearly that no significant contributing effect occurs to either quantitative (DIC) or isotopic results. The quantitative differences in DOC (ppmC) are due to sample size vs. calibration and not to the analysis. The difference between the DIC and DOC concentrations requires that the analyses be done on two independent aliquots. Surprisingly, the DOC isotopic values are very close for both the 1-mL (0.4 V IRMS signal) and 5-mL (2–3 V IRMS signal) aliquots, showing remarkable instrument isotopic linearity.

Speleothems are another interesting application using this instrument. Here samples from a cave near Perkins, Québec, show some interesting data comparisons between ceiling percolation and a running spring across the cave floor during three different times of year. Four of these samples were analysed for both species of carbon, and a second analysis was performed by dilution with deionised water. Again the reproducibility was well within the acceptable precision and accuracy. Further discussion of these samples forms part of a larger project led by Dr Bernard Lauriol, studying the formation of Moonmilk in cave systems.

Cross-contamination between DOC and DIC

A relevant question concerns how well the system performs when compared with dual-inlet analysis for DIC. Mixes of real samples were run for comparison, and the results are shown in Table 7. Note that the DIC samples were run 2 weeks after the dual-inlet off-line preparation. Some of the DIC delta differences could be due to opening and closing of sample bottles, as well as to the headspace created by the initial sample preparation and long storage time. The initial field samples were stored in 250-mL borosilicate brown bottles sealed with a polymer-coned screw cap. The samples were chosen to obtain varied isotopic compositions. Aliquots of samples were poured from the bottles into 60-mL syringes and injected into a 500-mL vacuum reaction flask through a silicone septum (Fig. 4). Phosphoric acid was then used to react with the DIC to produce CO_2.

The acid can either be at the bottom of the reaction vessel or in a side arm (multiple-use vessels). The amount of sample

Table 7. Comparison of DIC analysis by dual-inlet and TOC analyser. Each sample was analysed as is. Second aliquots of five of the samples were spiked with DOC to determine whether any effect on the DIC was apparent. RS = Raw sugar at 10 ppmC ($\delta^{13}C_{PDB} = -11.86‰$) Atrp = Atropine at 10 ppmC ($\delta^{13}C_{PDB} = -28.13‰$) Sucrose at 20 ppmC ($\delta^{13}C_{PDB} = -11.55‰$)

	DIC			DOC
Samples #	Dual-inlet $\delta^{13}C_{PDB}$(‰)	TOC Anal. $\delta^{13}C_{PDB}$(‰)	Difference (TOC)–(DI) (‰)	$\delta^{13}C_{PDB}$(‰)
B00-104-028	−4.83	−5.20	−0.37	−20.46
B00-104-028 + RS		−5.18	−0.35	−13.94
B00-104-029	−16.10	−15.91	0.19	−26.00
B00-104-029 + RS		−15.99	0.09	−15.92
B00-104-030	−15.84	−15.63	0.21	−25.29
B00-104-030 + Atrp		−15.82	0.02	−27.73
B00-104-031	−15.59	−15.54	0.05	−25.14
B00-104-031 + sucrose		−15.50	0.09	−14.31
B00-104-033	−8.09	−8.54	−0.45	−18.37
B00-104-033 + Atrp + RS		−8.50	−0.41	−21.23
B00-104-041	−4.59	−4.74	−0.15	—
Ammonium bicarbonate	−3.44	−3.18	0.26	—

used depends on the alkalinity, and varies from 10 to 150 mL in order to obtain about 50 μmol of CO_2 for a regular dual-inlet run. The gas is then cryogenically purified and quantitatively measured on a vacuum extraction line. The $\delta^{13}C_{PDB}$ of the CO_2 was measured using a triple collector VG-SIRA12 isotope ratio mass spectrometer. The comparison between dual-inlet and the TOC-IRMS results clearly show that the instrument is performing well within analytical precision for DIC, and the reproducibility is excellent. The DOC is shown as an indicator of direction in the isotopic shift created by the spike for the first five samples.

Dilution experiments in the laboratory have shown that high accuracy isotopic mixes can be achieved using the concentration-volume equation:

$$M_1V_1 = M_2V_2$$

where $M = \delta^{13}C_{PDB}$ in ‰ and V = volume in mL.

Further experiments were conducted to determine whether a DIC spike would have an effect on the DOC. It turned out that the products chosen, ACS reagent grades of sodium carbonate, sodium bicarbonate and ammonium bicarbonate, were actually contaminated with enough organic carbon to be detectable and make the experiment useless. This shows that when inorganic, and in some cases organic, products are used in an experiment, it becomes important to check the background for carbon contamination.

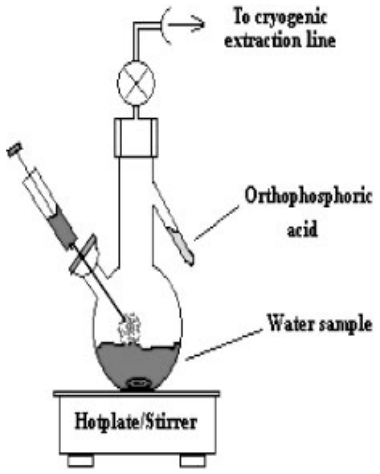

Figure 4. Off-line DIC extraction vessel.

CONCLUSIONS

We believe this to be the first successful use of a TOC analyser for both dissolved inorganic and, specifically, dissolved organic species for ^{13}C stable isotope analysis in an automated continuous-flow IRMS system. The analysis is relatively simple with little or no sample manipulation with a routine precision similar to that of a EA-IRMS, with a $\delta^{13}C_{PDB}$ standard deviation equal to or better than 0.2‰. Like the elemental analyser, it is still necessary to have an idea of initial concentration for targeting within the linear or dynamic range of the IRMS. An initial quantitative run is often recommended for concentration evaluation followed by IRMS targeting, either by sample dilution with DIW or by varying the aliquot size (1–25 mL). For DIC, alkalinity is often known and quantitative information can be derived from this for sample preparation.

The use of this system opens many new avenues for organic carbon analysis. For example, the metabolic pathways for organic compounds produced and consumed by aerobic and anaerobic bacterial biodegradation could be studied. A special high-pressure liquid chromatography (HPLC) system is being used for compound-specific separation of various dissolved organic mixtures, with a subsequent automated LC fraction collector that has the ability to capture and concentrate purified dissolved organic components in individual vials, thus increasing the output and precision of the preparation. Individual compounds are then analysed on the TOC-IRMS system. ^{13}C-Spiked organic compounds can be used in natural and *in vivo* experiments for tracer studies. Other possibilities that are being explored include the following: (a) with the proper chemistry, the system could potentially be used as an automated reaction chamber for converting nitrogen compounds such as nitrates or nitrites to N_2 gas for IRMS analysis; (b) dissolved gases such as N_2 or O_2 could be studied for isotopes, and potentially quantitative

analysis, using the sparging capabilities and reaction chamber temperature control of the TOC analyser; (c) VOC could be sparged out, and indeed preliminary tests on 40% alcohol products such as whiskey or vodka showed fairly good matches to off-line or GC headspace results; however, this analysis is best performed using a GC/combustion-IRMS system[13] with gas headspace capabilities.

It is believed that this technique will allow new and exciting studies for stable isotope research in both natural abundance and organic tracer studies not easily achieved before. Use of the TOC-IRMS is now a routine analytical process at the University of Ottawa.

Acknowledgements

Special thanks go to the G.G. Hatch Isotope Laboratories personnel, Paul Middlestead, Wendy Abdi and Patricia Wickham, for their support in getting this research off the ground and taking over some administrative pressures; to Graham Hughes of Graden Instruments, Canada, for his help in proving that this was indeed possible by providing another customer's instrument for 24 hours to hook up and test my theory; to Chuck Douthitt, Finnigan-MAT US, for believing in this enough to convince OI Analytical that this was a viable analytical technique; to OI Analytical Instruments US, for the modifications to the instrument hardware and software allowing the IRMS trigger for timing of the analysis; to Dr Ian D. Clark for his support and discussions and his embracing of the technique as a powerful tool to further his research; to Dr Bernard Lauriol for the use of samples from his Moonmilk project; to Dr Serge Occhietti, UQAM, for support and sharing of ideas throughout the years.

REFERENCES

1. Aravena R, Schiff SL, Trumbore SE, Dillon PJ, Elgood R. *Radiocarbon* 1992; **34**(3): 1.
2. Barrie A, Lemley M. *Am. Lab*. 1989; **21**(8): 54.
3. Clark ID, Fritz P (eds). *Environmental Isotopes in Hydrogeology*, Lewis Publishers, 1997; 328.
4. Hessen DO, Tranvik LJ (eds). *Aquatic Humic Substances: Ecology and Biogeo-chemistry*; Ecological Studies, vol. 133, ISBN 3-540-63910, Springer: Heidelberg, 1998.
5. Le Clercq M, van der Plicht J, Meijer HAJ. *Anal. Chim. Acta* 1998; **370**: 19.
6. OI Analytical, Application notes, #05570396; Presented at Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy 1996.
7. OI Analytical, Application notes, #10450497; Presented at Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy 1997.
8. Owen DM, Pirnie M, Amy GL. Characterization of Natural Organic Matter and Its Relationship to Treatability; Prepared by, University of Colorado; and Zaid K. Chowdhury, Malcolm Pirnie, Inc, July 1993, AWWA Research Foundation Order Number: 90631. Available: http://www.awwarf.com/exsums/90631.htm.
9. Rao RR, Killey RWD. *Radiochim. Acta* 1994; **65**: 63.
10. Schiff SL, Aravena R, Trumbore SE, Dillon PJ. *Water Resour. Res.* 1990; **26**: 2949.
11. *Stable Isotopes and Plant Carbon-Water Relations*, Ehlringer JR, Hall AE, Farquhar GD (eds). Physiological Ecology Series; ISBN 0-12-233380-2, 1993.
12. Wassenaar L, Aravena R, Fritz P. *Org. Geochem*. 1990; **15**: 383.
13. Preston T, Owen NJP. *Biomed. Mass Spectrom*. 1985; **12**: 510.
14. *Carbon Isotope Techniques*, Coleman DC, Fry B (eds). Isotope Techniques in Plant, Soil and Aquatic Biology Series, ISBN-0-12-179731-7, 1991.
15. Hood EW, Williams MW. Seasonal changes in the character and nitrogen content of dissolved organic material in an alpine/subalpine headwater catchment. In *Optimizing Nitrogen Management in Food and Energy Production and Environmental Protection*. Proceedings of the 2nd International Nitrogen Conference on Science and Policy. *The Scientific World* 1; 2001.
16. Aiken GR, McKnight DM, Thorn KA, Thurman EM. *Org. Chem*. 1992; **18**: 567.
17. Aiken GR. *Environ. Sci. Technol*. 1992; **26**: 2435.
18. St-Jean G. ^{13}C analysis of DIC and DOC in CF-IRMS using a TOC analyzer, Applied Isotope Geochemistry IV Conference, Pacific Grove, California, 2001, Extended abstract, PDF format: Available. http://wwwrcamnl.wr.usgs.gov/kinwater/default.htm.
19. Available: http://www.isotopes.science.uottawa.ca/services/services.htm.