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Determination of the $\delta(^{15}N/^{14}N)$ and $\delta(^{13}C/^{12}C)$ of Total N and C in Solids: RSIL Lab Code 1832

Chapter 5 of
Book 10, Methods of the Reston Stable Isotope Laboratory
Section C, Stable Isotope-Ratio Methods

Techniques and Methods 10-C5

U.S. Department of the Interior
U.S. Geological Survey

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DIRK KEMPTHORNE, Secretary

U.S. Geological Survey
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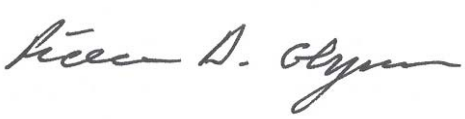
Foreword

The Reston Stable Isotope Laboratory (RSIL) provides stable isotope analyses on a routine basis for a large user community within the U.S. Geological Survey (USGS) and elsewhere. The RSIL also serves the USGS National Research Program (NRP) through its project on Stable Isotope Fractionation in Hydrologic Processes. The NRP conducts basic and problem-oriented hydrologic research in support of the mission of the USGS. The stable isotope project conducts research on the use of isotope-ratio measurements in studies of water resources and environmental quality. One objective of this project is to develop new techniques for isotopic analysis of H, N, O, C, and S in environmental samples. New analytical techniques expand the range of tools available for studying the movement of those elements in hydrologic and biogeochemical systems. Another objective of the project is to test new applications of isotope measurements in specific field settings. Field studies of isotope behavior have contributed to understanding of water-supply sustainability, ground-water/surface-water interactions, paleoclimate history, biologic cycling of nutrients, ground-water contamination, and natural remediation. This project also contributes to the improvement of measurement science and the development of isotope databases.

Book 10 of the Techniques and Methods Series of the USGS contains detailed descriptions of methods of the RSIL, including routine analytical methods called standard operating procedures (SOPs), along with safety guidelines, maintenance procedures, and other information about the operation of the RSIL. Section C of Book 10 contains the SOPs for a variety of methods to measure stable isotope ratios, each of which constitutes a chapter. Each chapter is limited to a narrow field of subject matter to permit flexibility in revision as the need arises.



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Conversion Factors

Multiply	By	To Obtain
	Length	
millimeter (mm)	0.03937	inch
centimeter (cm)	0.3937	inch
meter (m)	3.281	foot
	Volume	
micro liter (μL)	0.0610210^{-3}	cubic inch
milliliter (mL)	0.06102	cubic inch
cubic centimeter (cm3)	0.06102	cubic inch
	Mass	
nanogram (ng) = 10^{-3} μg	3.527×10^{-11}	ounce
microgram (μg) = 10^{-3} mg	3.527×10^{-8}	ounce
milligram (mg) = 10^{-3} g	3.527×10^{-5}	ounce
gram (g) = 10^{-3} (kg)	3.527×10^{-2}	ounce
kilogram = 10^{3} g; 10^{6} mg; 10^{9} μg; 10^{12} ng	2.205	pound
	Density	
gram per cubic centimeter (g/cm^3)	0.5780	ounce per cubic inch
	Temperature	
Celsius (°C)	°F = 9/5 (°C) + 32	Fahrenheit (°F)
	Pressure (force/area)	
kilopascal (kPa)	9.869×10^{-3}	atmosphere, standard (atm)
kilopascal (kPa)	1.450×10^{-1}	pound-force/square inch (psi)
kilopascal (kPa)	1.000×10^{-2}	bar
kilopascal (kPa)	2.961×10^{-1}	inches of mercury at 60°F

Acronyms and Abbreviations

ANSI	American National Safety Institute
ASR	analytical services request
CF-IRMS	continuous flow isotope-ratio mass spectrometer
DIW	deionized water
EA	elemental analyzer
GC	gas chromatograph
HDPE	high-density polyethylene
IAEA	International Atomic Energy Agency
IRMS	isotope-ratio mass spectrometer
LIMS-LSI	Laboratory Information Management System for Light Stable Isotopes

mg/g	milligram per gram
MSDS	Material Safety Data Sheets
NBS	National Bureau of Standards
NFPA	National Fire Protection Association
NWQL	National Water-Quality Laboratory
PCM	polycarbonate membrane
per mill, ‰	1/1000
pF	picofarad
QA	quality assurance
QC	quality control
QC/QA	quality control/quality assurance
RSIL	Reston Stable Isotope Laboratory
SOP	standard operating procedure
USGS	U.S. Geological Survey
VPDB	Vienna Peedee Belemnite

Symbols

Ω	ohm
<	less than
>	greater than
≥	greater than or equal
≤	less than or equal

Determination of the $\delta(^{15}N/^{14}N)$ and $\delta(^{13}C/^{12}C)$ of Total N and C in Solids: RSIL Lab Code 1832

By Kinga Révész and Haiping Qi

Summary of Procedure

The purpose of Reston Stable Isotope Laboratory (RSIL) lab code 1832 is to determine the $\delta(^{15}N/^{14}N)$, abbreviated as $\delta^{15}N$, and the $\delta(^{13}C/^{12}C)$, abbreviated as $\delta^{13}C$, of total nitrogen and carbon in a solid sample. A Carlo Erba NC 2500 elemental analyzer (EA) is used to convert total nitrogen and carbon in a solid sample into N_2 and CO_2 gas. The EA is connected to a continuous flow isotope-ratio mass spectrometer (CF-IRMS), which determines the relative difference in stable nitrogen isotope–amount ratio ($^{15}N/^{14}N$) of the product N_2 gas and the relative difference in stable carbon isotope–amount ratio ($^{13}C/^{12}C$) of the product CO_2 gas. The combustion is quantitative; no isotopic fractionation is involved. Samples are placed in tin capsules and loaded into a Costech Zero-Blank Autosampler on the EA. Under computer control, samples then are dropped into a heated reaction tube that contains an oxidant, where combustion takes place in a He atmosphere containing an excess of oxygen gas. Combustion products are transported by a He carrier through a reduction furnace to remove excess oxygen and to convert all nitrous oxides into N_2 and through a drying tube to remove water. The gas-phase products, mainly CO_2 and N_2, are separated by a gas chromatograph (GC). The gas is then introduced into the IRMS through a Finnigan ConFlo II interface. The ConFlo II interface is used for introducing not only sample into the IRMS but also N_2 and CO_2 reference gases and He for sample dilution. The flash combustion is quantitative; no isotopic fractionation is involved. The IRMS is a Finnigan Delta^{Plus} CF-IRMS. It has a universal triple collector, two wide cups with a narrow cup in the middle; it is capable of measuring mass/charge (*m/z*) 28, 29, 30 or with a magnet current change 44, 45, 46, simultaneously. The ion beams from these *m/z* values are as follows: *m/z* 28 = N_2 = $^{14}N^{14}N$; *m/z* 29 = N_2 = $^{14}N^{15}N$ primarily; *m/z* 30 = NO = $^{14}N^{16}O$ primarily, which is a sign of contamination or incomplete reduction; *m/z* 44 = CO_2 = $^{12}C^{16}O^{16}O$; *m/z* 45 = CO_2 = $^{13}C^{16}O^{16}O$ primarily; and *m/z* 46 = CO_2 = $^{12}C^{16}O^{18}O$ primarily.

Reporting Units and Operational Range

Variations in stable isotope–amount ratios typically are small. Stable isotope–amount ratios commonly are determined as the relative difference in the ratio of the amount of the less abundant isotope (usually the heavy isotope) to the amount of the more abundant isotope (usually the light isotope) of the sample with respect to the reference. This relative difference is designated $\delta(^{i}E)$, which is a shortened form of $\delta(^{i}E/^{j}E)$ or $\delta(^{i}E)$ and is defined according to equation 1:

$$\delta^{i}E = \delta\left(^{i}E\right) = \delta\left(^{i}E/^{j}E\right) = \frac{n_X\left(^{i}E\right)/n_X\left(^{j}E\right) - n_{ref}\left(^{i}E\right)/n_{ref}\left(^{j}E\right)}{n_{ref}\left(^{i}E\right)/n_{ref}\left(^{j}E\right)} \quad \textbf{(1)}$$

where *i* and *j* are isotopes of element E and $n_X(^{i}E)/n_X(^{j}E)$ and $n_{ref}(^{i}E)/n_{ref}(^{j}E)$ are the ratios of the isotope amounts in the unknown X and a reference ref. A positive $\delta^{i}E$ value indicates that the unknown is more enriched in the amount of the heavy isotope ^{i}E than the reference. A negative $\delta^{i}E$ value indicates that the unknown is depleted in the amount of the heavy isotope relative to the reference. Values of $\delta^{i}E$ have been

reported in parts per hundred (% or percent), parts per thousand (‰ or per mill), and parts per ten thousand. For stable nitrogen isotope–amount ratios, $\delta^{15}N$ is defined as follows:

$$\delta^{15}\mathrm{N} = \delta\left({}^{15}\mathrm{N}\right) = \delta\left({}^{15}\mathrm{N}/{}^{14}\mathrm{N}\right) = \frac{n_X\left({}^{15}\mathrm{N}\right)/n_X\left({}^{14}\mathrm{N}\right) - n_{ref}\left({}^{15}\mathrm{N}\right)/n_{ref}\left({}^{14}\mathrm{N}\right)}{n_{ref}\left({}^{15}\mathrm{N}\right)/n_{ref}\left({}^{14}\mathrm{N}\right)} \quad \textbf{(2)}$$

For stable carbon isotope ratios, $\delta^{13}C$ is defined as follows:

$$\delta^{13}\mathrm{C} = \delta\left({}^{13}\mathrm{C}\right) = \delta\left({}^{13}\mathrm{C}/{}^{12}\mathrm{C}\right) = \frac{n_X\left({}^{13}\mathrm{C}\right)/n_X\left({}^{12}\mathrm{C}\right) - n_{ref}\left({}^{13}\mathrm{C}\right)/n_{ref}\left({}^{12}\mathrm{C}\right)}{n_{ref}\left({}^{13}\mathrm{C}\right)/n_{ref}\left({}^{12}\mathrm{C}\right)} \quad \textbf{(3)}$$

The primary reference material for relative nitrogen isotope-ratio measurements ($\delta^{15}N$) is atmospheric nitrogen gas (N_2), which is widespread and homogeneous, and, by convention, has a $\delta^{15}N$ consensus value of 0 ‰ (Mariotti, 1983). The primary reference materials for relative carbon isotope-ratio measurements ($\delta^{13}C$) are National Bureau of Standards (NBS) 19 calcium carbonate and L-SVEC lithium carbonate, which have consensus $\delta^{13}C$ values of +1.95 ‰ and –46.6 ‰ on the Vienna Peedee Belemnite (VPDB) scale (Hut, 1987; Coplen, 1996; Coplen and others, 2006). By interspersing internationally distributed isotopic reference materials with accepted δ values among unknown samples, the δ values can be determined on normalized $\delta^{15}N$ and $\delta^{13}C$ scales. The $\delta^{15}N$ and $\delta^{13}C$ measurements are made with a CF-IRMS, which alternately measures the isotope–amount ratios of the sample N_2 and CO_2 gases and one or more injections of the "working reference" N_2 and CO_2 gases. Values of $\delta^{15}N$ and $\delta^{13}C$ typically are reported in parts per thousand (‰).

The system was tested by analyzing pure inorganic samples (potassium and sodium nitrate, ammonium sulfate, and calcium and lithium carbonate), an array of biological tissues from vertebrates and invertebrates, and geological samples, such as suspended organic matter in sediment (Révész, 1998). The routine analysis requires a minimum of 40 µg of sample as nitrogen and 40 µg of sample as carbon in a maximum of 40 mg of solid sample; however, the system is capable of analyzing samples as small as 19 µg of N and 19 µg C in one sample aliquot. The $\delta^{15}N$ values of reference materials ranged from about 5 to +180 ‰, whereas $\delta^{13}C$ varied from about –26 to +38 ‰, which covers much of the range of isotopic composition of natural samples. The precision and accuracy of the results were acceptable (±0.2 ‰ for both $\delta^{13}C$ and $\delta^{15}N$ measurements) and no memory effects were observed (Qi and others, 2003).

Reference Materials and Documentation

Reference Materials Used, Storage Requirements, and Shelf Life

In this method, L-glutamic acid ($HO_2CCH_2CH_2CH(NH_2)CO_2H$) internationally distributed isotopic reference materials USGS40 and USGS41 are used because they contain both nitrogen and carbon. USGS40 has a $\delta^{15}N$ value of –4.52 ‰ and a $\delta^{13}C$ value of –26.24 ‰. USGS41 has a $\delta^{15}N$ value of +47.57 ‰ and a $\delta^{13}C$ value of +37.76 ‰ (Qi and others, 2003; Coplen and others, 2006). Both of these reference materials are stored in Reston in the RSIL. Their shelf life is indefinite.

Documentation

All calibration results are stored in the Laboratory Information Management System for Light Stable Isotopes (LIMS-LSI) (Coplen, 2000) under the following sample identifiers: G-8396 and G-8397.

Labware, Instrumentation, and Reagents

Preparatory labware and apparatus include silver and tin capsules and a microbalance capable of measuring samples with 0.0001-mg precision. The analytical apparatus consists of four different units: (1) EA, (2) ConFlo interface, (3) IRMS, and (4) computer software.

The EA is a Carlo Erba Instruments NC 2500 (CE NC 2500) system, and it is equipped with a Costech "zero blank" autosampler that holds 49 samples enclosed in tin capsules (fig. 1). Each tin capsule falls into the reaction-tube, which is kept at 1020 °C and is under constant He flow (90 mL/min). The sample immediately reacts with a measured amount of oxygen released from a 5-mL loop purged at 35 mL/min. The reaction of oxygen with the tin capsule is exothermic, which results in localized temperatures of up to 1800 °C, thus ensuring complete and instantaneous sample combustion. This reaction is called "dynamic flash combustion." The combustion gases are first carried by He through an oxidative catalyst layer (Cr_2O_3) in the reaction tube, where oxidation is completed, and then through a reduction agent (Cu) in another reaction tube kept at 650 °C where any nitrogen oxides are converted into elemental nitrogen and any excess oxygen is consumed. At the outlet of the second reaction tube, the gas mixture (N_2, CO_2, and H_2O) enters a trap that contains Anhydrone, which absorbs water. The gas mixture, now N_2 and CO_2, flows through a GC column (Poraplot PQS kept at 50 °C), which separates the gases, screens out sampling noise, and ensures that pure nitrogen and carbon dioxide gas pass separately through the thermal conductivity detector, through the Conflo II open split, and into the IRMS.

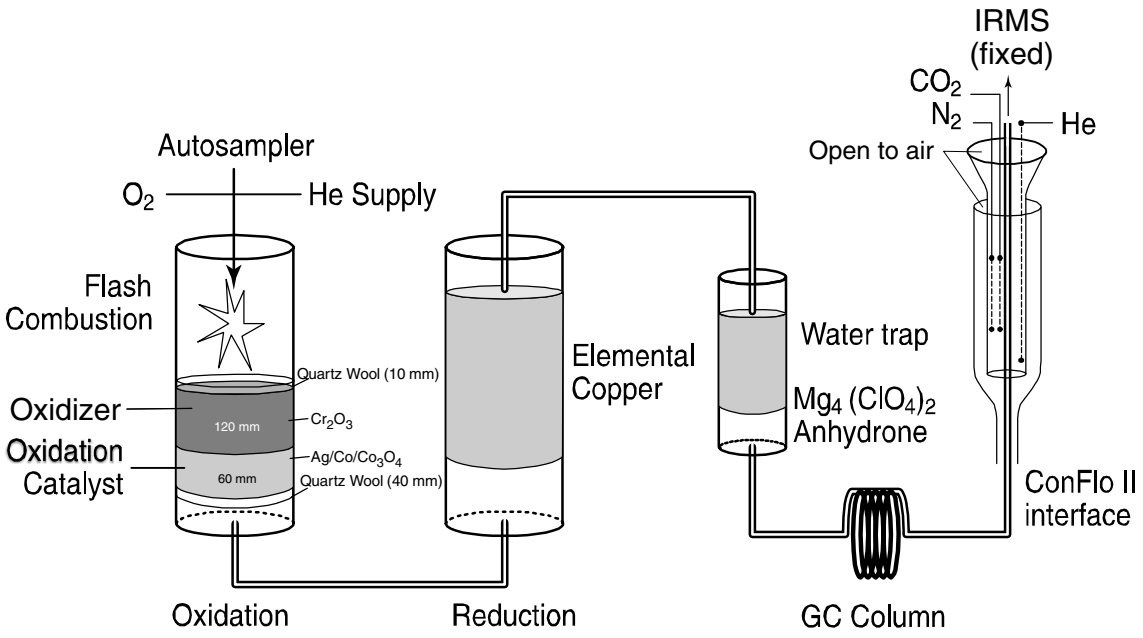

Figure 1. Diagram of the Carlo Erba Instruments NC 2500 E (CE NC 2500) Elemental Analyzer (used with permission from Thermo Electron Co.).

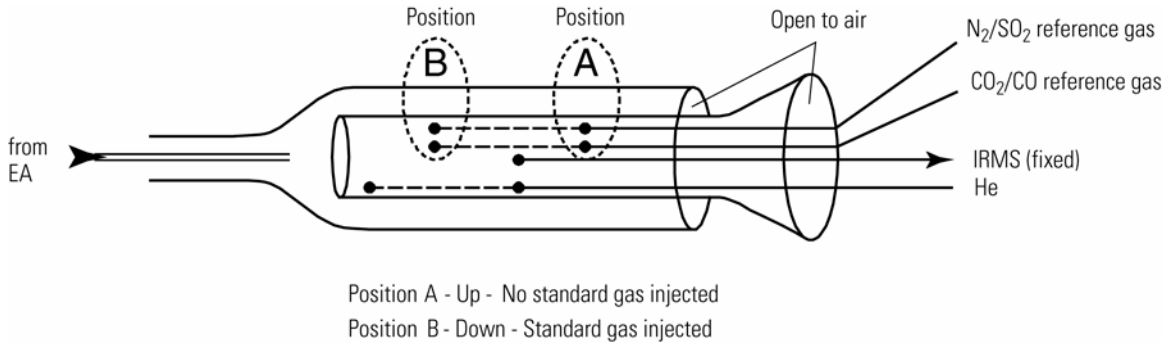

Figure 2. Diagram of a Finnigan ConFlo II Open Split (used with permission from Thermo Electron Co.).

The Finnigan ConFlo II provides the means for coupling the EA to the CF-IRMS with an open-split arrangement (fig. 2). The principle of an open split is that it reduces the 90 mL/min He flow needed to operate the EA to a 0.3 mL/min He flow needed to operate the IRMS. This is achieved by placing the end of the capillary that leads to the IRMS directly in the flow of He coming from the EA. The narrow diameter and length of this capillary limit the flow of gas to the IRMS. Furthermore, extra capillaries carrying reference gas or He gas can be added to the open split, thereby making it possible to inject reference gas into the IRMS or to dilute sample with extra He if the sample is too large (or to direct gases away by dilution if they are too large to ensure total separation).

The IRMS is a Finnigan Delta^{Plus} CF-IRMS (fig. 3). The fundamental principle of the CF-IRMS technique is that a carrier gas transports the analyte through an initial stage of online chemistry for conversion to a form acceptable by the IRMS (Brenna and others, 1997). This is an automated system generating online, high-precision, δ values of bulk solid and nonvolatile liquid samples. In the IRMS, gas molecules are ionized in a source by electrons emitted from a hot filament. The ions are accelerated into an analyzer, separated in a magnetic field, and collected in Faraday cup collectors. The ion-beam intensities are measured with electrometers. This IRMS has a universal triple collector, two wide cups with a narrow cup in the middle, capable of measuring *m/z* 28, 29, and 30 simultaneously or, with a magnet current change, *m/z* 44, 45, and 46 simultaneously. The resistor–capacitor combination on the electrometers used to measure these masses are as follows: 3×10^8 Ω and 680 pF for cup 1; 3×10^{10} Ω and 5 pF for cup 2; 3×10^{11} Ω and 2 pF for cup 3.

The Finnigan ISODAT 2.0 software is designed (1) to advance the autosampler carousel, (2) to control the ConFlo II interface to inject reference gases at the desired time and dilute the sample if desired, (3) to operate the IRMS, (4) to acquire data from the IRMS, and (5) to calculate delta values.

Sample Collection, Preparation, Analysis, Retention Times, and Disposal

Sample Containers, Preservation, and Handling Requirements

Each sample is collected in a high-density polyethylene (HDPE) scintillation vial with Polyseal cap, Wheaton 986706 available from the RSIL . Sample size should be a maximum of 1 g. The sample should be homogenized, oven dried (90 °C), and powdered by the submitter to 100 to 200 µm. Containers are labeled with isotopes, whose concentrations are to be determined, and with respective laboratory code or

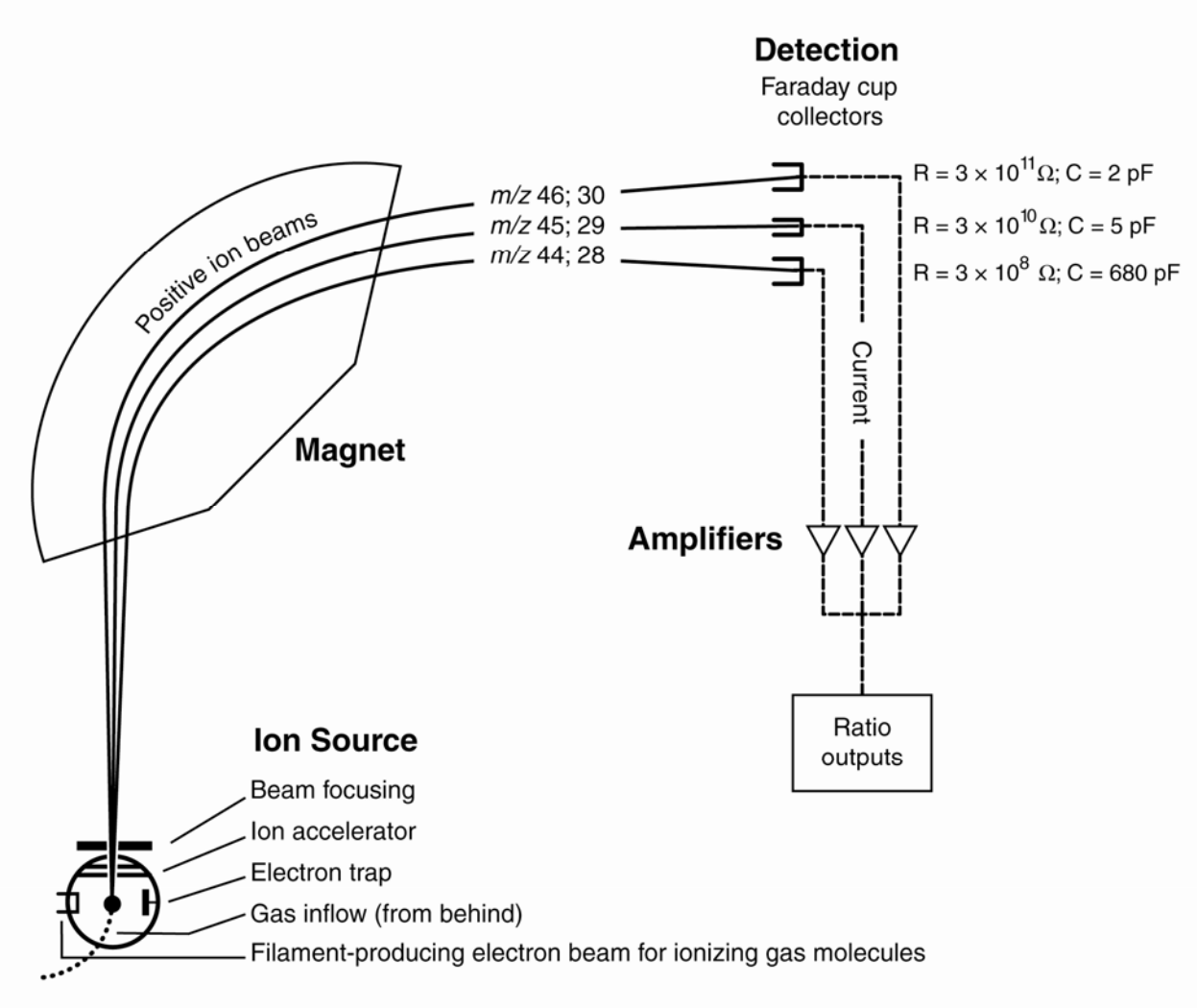

Figure 3. Schematic of a continuous flow isotope-ratio mass spectrometer (CF-IRMS) (modified from Clark and Fritz, 1997).

schedule number. A minimum of 0.15 mg of nitrogen per sample and 0.15 mg of carbon per sample are required. The minimum sample concentration is 1 mg/g sample for nitrogen and 1 mg/g sample for carbon. Lower concentrations can be analyzed; contact the laboratory for further information. No treatment, preservation, or special shipping is required.

Sample Preparation and Time Requirements

Sample preparation involves logging in samples to the LIMS-LSI, creating a sample work order sheet, and weighing samples into capsules.

The logging-in procedure involves logging samples in batches to LIMS-LSI that could contain from 1 to 100 samples per batch (also called a project), printing labels for each sample, and printing a summary project report. Each sample label contains the Field ID (the identifier by which the sample submitter knows each sample) and the unique RSIL Lab ID assigned by the LIMS-LSI. The summary project report is inserted into a three-ring binder titled “Samples in Progress.”

If samples are submitted through the USGS National Water Quality Laboratory (NWQL), the Field ID is a unique number for each sample assigned according to NWQL guidelines; this unique number is written on the analytical services request (ASR) form accompanying a sample, and the ASR information is entered into the LIMS-LSI system. This information is e-mailed to the submitter of the samples for identification of any errors and subsequently e-mailed to NWQL. Copies of the ASR forms are mailed to NWQL. Step-by-step procedures are given in Appendix A.

An Excel sample workbook is generated. The step-by-step procedures and examples are shown in Appendix B. Sample weighing includes balance conditioning, weighing sufficient sample to yield 40 µg of C and 40 µg of N into capsules, closing capsules by folding them, and recording weight on template. Step-by-step procedures are given in Appendix C. The time requirement for the sample preparation is about 1 to 5 days.

Performing the Analysis and Time Requirements

All samples are prepared and analyzed in batches of a maximum of 80 per day, and most samples are analyzed in duplicate. Six aliquots of two internationally distributed isotopic reference materials with different isotopic compositions are analyzed at the beginning of a batch. Four are depleted in ^{13}C and ^{15}N, and two are enriched in ^{13}C and ^{15}N. The reference materials depleted in ^{13}C and ^{15}N also are interspersed among the samples. No fewer than one reference material for every ten unknowns is analyzed. The amount of nitrogen in the reference materials should be in the same range as that of the samples, and the CO_2 is diluted accordingly. Therefore, one must preanalyze every sample to obtain the C/N concentration ratio unless it is provided by the customer. If the C/N ratio is greater than nine, the normalization against reference material analyzed with the samples is not reliable; therefore, one must analyze N and C isotope ratios separately. If the C/N concentration ratio is unknown and cannot be determined prior to isotopic analysis, a sequence of reference materials containing different amounts must be analyzed to determine a mass dependent correction factor.

The procedure involves loading the autosampler carousel with capsules containing an appropriate amount of sample in sequence according to the "Template" (Appendix D), loading a new sample heading (including sample weights) into the "Sequence Table" in ISODAT 2.0, choosing the appropriate "Method," and starting sequence acquisition (Appendix E). After the analyses are completed, the data are exported (Appendix F) to a ZIP disk or "memory stick" from the ISODAT 2.0 database and imported (Appendix G) to the LIMS-LSI and transferred to the Data Back-Up computer. Final daily correction factors are determined with LIMS-LSI (Appendix H), based on the daily analyses of reference materials using equations 4 and 5 below, which are presented with numerical values:

$$-4.52\ ‰ = m \times \delta^{15}N_{\text{USGS40 - workingref.gas}} + b \quad \textbf{(4)}$$

$$+47.57\ ‰ = m \times \delta^{15}N_{\text{USGS41 - workingref.gas}} + b \quad \textbf{(5)}$$

The values -4.52 ‰ and +47.57 ‰ are the assigned values of the two international reference materials (USGS40 and USGS41, respectively). The $\delta^{15}N_{\text{ref – workingrefgas}}$ values in equations 4 and 5 are the mean daily delta values of the respective international reference materials relative to the working reference gas, b is the additive correction factor, and m is the expansion coefficient correction factor. Carbon isotopic correction factors are determined in the same way; however, the values of the carbon isotopic internationally distributed reference materials should be used, and the $\delta^{13}C$ values in the equations would be the daily delta values of the respective internationally distributed reference materials relative to the working reference gas. When two or more reference materials with different isotopic compositions are analyzed, the correction for sample blank does not need to be applied if the magnitude of the blank in the samples and reference materials is identical. However, if only a single reference

material is analyzed, only one-point calibration is possible; therefore, a blank should be interspersed with unknowns and a blank correction must be applied.

If replicates do not agree within acceptable tolerances, they are analyzed until acceptable statistics are achieved. The computerized LIMS-LSI will not release data until the statistics are acceptable. The time required for performing the analyses is a minimum of 2 days.

The systematic procedure is listed in the "Lab Procedures" binder at the RSIL. The supervisor reviews suggested changes before they are adopted.

Problematic Samples

Problematic samples include those that do not have nitrogen and carbon concentration ratios prior to isotopic analysis. In that case, the C/N concentration ratio needs to be determined by a preliminary EA analysis to be able to determine the sample weight that gives appropriate N and C amounts (40 µg each) for analysis and the appropriate dilution of with He, as necessary.

Interferences

There are no known interferences with this analytical technique. However, it should be pointed out that if samples contain a mixture of different nitrogen and carbon sources, reported isotope-ratio values represent the isotopic composition of the total nitrogen and carbon in the sample. The $\delta^{15}N$ or $\delta^{13}C$ values of specific C or N species cannot be provided with this analytical technique.

Troubleshooting and Bench Notes

The most common problems with this analytical technique are (1) incomplete combustion, (2) leakage in the system, particularly in the EA, and (3) insufficient linearity of the IRMS.

The fact that the melting point of the compounds being combusted are usually higher or close to the reaction-tube temperature (1020 °C) could create insufficient combustion. The "flash point" has a higher temperature, approximately 1800 °C (because the tin oxidation reaction is exothermic); therefore, it is critical to create a sharp, sudden, bright, single flash. This can be done by coordinating (1) He pressure and flow rate, (2) O_2 pressure and flow rate, and (3) sample "start time." The system is so sensitive that even a 1-s difference in the sample dropping time can make a difference in the brightness of the flash, peak shape, peak area, and, consequently, the δ result. The optimum set-up parameters of the RSIL EA are cycle = 100, oxy = 60 (O_2 flow stops flowing into autosampler), sample start = 10, and sample stop = 40. The helium regulator is set for 150 kPa, and He flow setup is 95 mL/min, which actually measures 120 mL/min at the vent. The oxygen regulator is set for 150 kPa, and the oxygen flow rate is 54 mL/min at purge. The oxygen loop is 5 mL. Oven temperature of the GC is 50°C.

Even a tiny leak can interfere with the result; it can create double peaks, an unstable analysis, and so forth. The high He flow rate makes the system more sensitive to leaks (Bernoulli principle). Leaks can cause uncontrollable changes in the flow rate, which could interfere in the quantitative combustion, in the peak shape, in the peak area, and consequently, in the δ values. Ash accumulation is also critical. Although tin oxide serves as a catalyst in the reaction, it also partially "clogs" the system, interfering with the flow rate, although not necessarily in a measurable manner. However, this assumes a nonrandom interference that causes the shift in δ values. By frequently analyzing reference materials, this shift can be corrected. However, after approximately 50 samples (one carousel), it is advisable to remove the ashes (Révész, 1998). The leak-checking procedure is given in Appendix I. A leak is indicated in the IRMS when m/z 28 and 29 are high and the ratios of m/z 28, 32, and 40 (N_2, O_2, and Ar) reflect atmospheric

compositions. Acceptable levels for *m*/*z* 28 and 29 are approximately 0.15 and 0.015 V, respectively, with typical IRMS settings. (See "Daily Checklist" in Appendix J.)

The IRMS must be focused carefully. The analyst should start with the original factory setting. In the RSIL, the settings (in priority order) are as follows: Extraction = 7.00; X focusing = 4.8; Voltage = 8.00; Trap = 5.7; Electron = 7.8; Y1 focusing = 3.00; Y2 focusing = 3.8; X deflection = 6.4; Extraction = 5.6; and X-focusing symmetry = 3. Emission current is critical. The higher it is, the higher the signal is but the poorer the linearity is. The IRMS is temperature sensitive; therefore, it must be operated in a temperature-controlled environment with all of the side panels on. The resistor–capacitor combinations on the electrometers used to measure m/z 28, 29, and 30 or m/z 44, 45, and 46 are 3×10^8 Ω and 680 pF for cup 1, 3×10^{10} Ω and 5 pF for cup 2, and 3×10^{11} Ω and 2 pF for cup 3.

An internal precision check needs to be conducted using the "Zero Enrichment" method (standard gas on/off 10 times), and it must give a standard deviation of ± 0.1 ‰ or better for both δ^{15}N and δ^{13}C values. The "Ratio Linearity" test must give 0.12 ‰/V or better linearity. An external precision check needs to be performed as needed, approximately every week or so; samples of the same amount need to be analyzed within ± 0.010 mg. The 1–σ standard deviation must be ±0.2 ‰ or better for n = 10. If either of these criteria has not been met, the IRMS cannot be used and must be refocused.

Maintenance and Maintenance Records

Routine maintenance is necessary for the upkeep of the IRMS vacuum system, including (1) checking the pump performance from time to time, (2) changing the pump oil, and (3) changing the oil cartridge in each turbomolecular pump at 6-month intervals. Pump conditions, such as date of oil changes, problems, and repairs, are listed in the pump database (file path C:\RSIL\vacuum pumps). Maintenance of the EA includes (1) changing the insertion tube every day (Appendix K), (2) changing the water trap about every 200 samples (Appendix L), (3) changing reaction tubes about every 500 analyses (Appendix M), and (4) cleaning the sample carousel every week. The IRMS also requires a daily check (Appendix J). A logbook is kept for each IRMS, where notes of maintenance checks, normal settings, problems, and repairs are listed.

Maintenance of ISODAT 2.0 includes importing (transferring) results from the ISODAT 2.0 database to the LIMS-LSI computer hard disk and the Data Back-Up computer hard disk (Appendix G).

Sample Retention Time and Disposal

Samples are retained in the RSIL for at least 4 months after reporting data. Samples are then discarded unless the submitter has requested that the samples be returned.

The ISODAT sample files from the IRMS computer are kept indefinitely on two different hard disks of the Data Back-Up computer. Paper reports are kept for a few weeks. Analytical results from the IRMS are exported to LIMS-LSI and kept indefinitely. No data are entered by hand, and no manual calculations are performed on the data.

Data Acquisition, Processing, Evaluation, Quality Control, and Quality Assurance

Laboratory Information Management System for Light Stable Isotopes (LIMS-LSI)

In the RSIL, the LIMS-LSI (Coplen, 2000) is used for data processing and evaluation. This system is a database program capable of (1) storing information about samples, (2) storing the results of mass

spectrometric delta values of samples after importing from the ISODAT database, (3) calculating analytical results using standardized algorithms stored in a database, (4) normalizing delta values using isotopic reference materials, and (5) generating templates for convenient sample placement to facilitate loading of automated IRMS sample preparation manifolds and carousel. With this system, the following can be ensured: (1) quality assurance (QA), (2) laboratory efficiency, (3) reduction of workload and errors owing to the elimination of retyping of data by laboratory personnel, and (4) a decrease of errors in data reported to sample submitters. This database provides a complete record of information on how laboratory reference materials have been analyzed and provides a record of what correction factors have been used. It provides an audit trail for the RSIL.

Quality-Control (QC) Samples

Samples are prepared and analyzed in batches—a maximum of 80 daily. Each batch contains approximately six reference materials at the beginning, four depleted in ^{13}C and ^{15}N and two enriched in ^{13}C and ^{15}N, and later in the batch one depleted in ^{13}C and ^{15}N for every ten samples. The last analysis of a batch is that of a reference material depleted in ^{13}C and ^{15}N. All of these reference materials serve as QC samples. The preparation and analytical cost of these isotopic reference materials is the responsibility of RSIL. Both RSIL and NWQL monitor these results. Quarterly, RSIL prepares a summery of results of these internationally distributed isotopic reference materials and make them available to NWQL.

Daily, the analyst (1) examines the computer printouts for indications of analytical problems, (2) determines the daily additive and expansion correction factors by the LIMS-LSI, and (3) applies correction factors to isotopic data. The values of these correction factors along with the date and range of analysis numbers are recorded manually in the laboratory "EA Book" even though the data are already in the LIMS-LSI. After printing the list of isotopic results from the "Table of Samples in Progress," the analyst reviews the results, determines which samples need to be analyzed a third time to achieve acceptable results (i.e., $\Delta\delta \leq 0.2$ ‰ for both isotopes).

Acceptance Criteria for All QC Samples

Acceptance criteria for QC samples are the same as acceptance criteria for the samples. The rules are as follows:

1. If standard deviation is ≤ 0.2 ‰, use mean delta.
2. If there are three or more analyses, delete the outlier and recalculate.
3. If standard deviation of this recalculation is ≤ 0.2 ‰, use mean from this recalculation.
4. If none of the above is the case, the result is not accepted and corrective action is required.

Corrective Action Requirements

If an analyst finds any problem with the daily reference sample data, the analyst contacts the supervisor. The trouble-shooting process will require an evaluation and reanalysis of certain samples to ascertain the origin of the problem.

If samples do not give satisfactory results after three or more separate analyses, the analyst averages all the data and report the mean value. Such analytical results are indicated with a comment, and the customer will be advised by e-mail or other means. If the sample was submitted through the NWQL, it is the responsibility of the customer to determine whether the data need to be qualified. If so, the NWQL will mark the result in USGS databases appropriately.

Responsible Parties for All QA/QC Functions for Procedures Covered in RSIL SOPs

The analyst, with supervisor approval, is responsible for qualifying data and notifying customers.

Data Management and Records

In addition to evaluating daily sample analyses, every week, an analyst evaluates the data project-by-project, reports results to the customers, and files final project data reports in the laboratory "Correspondence" binder (Appendix N).

Health, Safety, and Waste-Disposal Information

Applicable Health and Safety Issues

Personal Protection

Safety glasses and protective gloves are recommended whenever samples are handled, especially when the samples are of biological origin. For other precautions and safety procedures, consult Material Safety Data Sheets (MSDS), which are on file in the laboratory, and http://www.ilpi.com/msds/#Manufacturers provides links to MSDSs of most chemical companies.

Electrical Hazards

Electrical systems must conform to the National Electric Code, National Fire Protection Association Code (NFPA 70-1971), and the American National Standards Institute (ANSI) Code (C1-1971). Consult the U.S. Geological Survey's Safety and Environmental Health Handbook (U.S. Geological Survey, 2002).

Shock hazards exist inside the instruments. Only an authorized service representative or an individual with training in electronic repair should remove panels or circuit boards where voltages are greater than 20 V. The instruments require a third wire protective grounding conductor. Three-to-two wire adapters are unsafe for these instruments.

Chemical Hazards

The hazardous chemicals used in the process are Cr_2O_3 and Co_3O_4/Ag, which are used in the oxidation reaction tube. Sometimes the reaction tube cracks, and the packing material leaks out. When this happens, protective gloves and a full-face mask are required during the cleanup process. All the materials, including gloves and any cleaning towels, need to be collected in a plastic bag and disposed of as hazardous waste. All samples should be carefully inspected upon receipt for obvious indications of hazards.

Gas Cylinder Handling

Compressed gas cylinders must be handled and stored according to the Safety and Environmental Health Handbook (U.S. Geological Survey, 2002). Each cylinder must be (1) carefully inspected when received; (2) securely fastened at all times with an approved chain assembly or belt; (3) capped at all

times when not in use; (4) capped when transported; (5) transported only by a properly designed vehicle (hand truck); and (6) stored separately with other full, empty, flammable, or oxidizing tanks of gas, as appropriate.

Specific Waste-Disposal Requirements

Used reaction tubes containing Cr_2O_3, and Co_3O_4/Ag must be collected in a closed container and given to the safety, health, and environment officer for disposal.

Revision History

Publication Series and Series Number: Techniques and Methods 10-C5 (Book 10, Section C, Chapter 5)

Publication Title: Determination of the $\delta(^{15}N/^{14}N)$ and $\delta(^{13}C/^{12}C)$ of Total N and C in Solids: RSIL Lab Code 1832

Publication Authorship: Révész, Kinga, and Qi, Haiping.

First Version and Date of First Release: December 2006
Current Version and Date of Current Release: July 2007
Summary of Product Components

Component	Description	Last Revised In Publication Version	Date of Last Revision

List of Revisions (latest version first):

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Appendix A. Step-by-Step Procedure to Log-In Samples to LIMS-LSI

1. For samples submitted through NWQL:
   a. Match up information on sample bottles with information on Analytical Services Request (ASR) forms by spreading out ASR forms and placing each bottle on its respective ASR form. Check for broken bottles.
   b. Assign NWQL #s based on the Julian date of the Monday following the submission date. Check note on bulletin board for 'Next NWQL #.' Write NWQL #s on ASR forms, then update "Next NWQL #" on bulletin board.
   c. Use "New Project Log-in" in LIMS-LSI to assign G-#s; field IDs are the Station IDs. Submission date is the date samples are logged in.
   d. Enter all ASR information into LIMS-LSI, sample by sample.
   e. Print out two Project Reports, Labels (two for each sample), and Sample Range Forms.
   f. Put a label on a sample bottle and on the corresponding ASR form; crosscheck Field IDs between bottles and ASR forms.
   g. Make one photocopy of each ASR form.
   h. Enter G-# range into the appropriate template.
   i. Proofread information on Sample Range Forms against ASR forms (recruit help if necessary). Make corrections in LIMS-LSI, if necessary, and printout corrected Sample Range Forms. Initial and date both Project Reports to indicate that proofreading is complete.
   j. In LIMS-LSI, save NWQL Headings to a floppy diskette.
   k. In ISOTOPES e-mail on the common-use PC, copy headings into new message from diskette by opening headings-file in Notepad and copying/pasting to new message. Print copy of headings and e-mail to each project chief for information review, and NWQL [Attn: Jeanne Hatcher (jhatcher@usgs.gov)].
   1. Mail copies (not originals) of ASR forms and corrected Sample Range Forms to NWQL [National Water Quality Laboratory, Attn: Jeanne Hatcher, U.S. Geological Survey, P.O. Pax 25708, MS 407, Building 95, Denver Federal Center, Denver, CO 80225-0608 (Tel: 303-236-3481)]. Envelope should be mailed every Friday.
   m. Punch holes in original ASRs and put in "Central Lab" binder along with project reports and date on piece of tape. Punch holes in NWQL headings and other project report and place in "Samples-in-Progress" binder.
   n. Add samples to the Table of Samples to Be Analyzed in LIMS-LSI.
      i. Click the "Projects" button of LIMS-LSI.
      ii. Select the project that was logged in.
      iii. Click the "Templates" button.
      iv. Select "EA C+N."
      v. Click "Add" and close LIMS-LSI.
   o. Put samples in cabinets for storage until analyzed.
2. For samples submitted directly to RSIL:
   a. For Sample submitter:
      i. Download "RSIL Excel Worksheet" from RSIL website at http://isotopes.usgs.gov/.
      ii. Fill out the requested sample information.
      iii. Send diskette and a hard copy along with the samples or e-mail the completed RSIL Excel worksheet to isotope@usgs.gov.
   b. For RSIL personnel:
      i. Match up information on sample bottles with submitted "Excel Worksheet."
      ii. Enter all Excel Worksheet information into LIMS-LSI by loading diskette. Submission date is the date samples are logged in.

iii. Use “New Project Log-in” in LIMS-LSI to assign G#s; field IDs are the Station IDs.
iv. Print out one Project Reports and container labels (one for each sample).
v. Put a label on a sample bottle and crosscheck Field IDs between bottles and Excel Worksheet forms.
vi. Enter G-# range into the appropriate template.
vii. Punch holes in original Excel Worksheet and all the information of the project you have, and put in “Samples-in-Progress” binder.
viii. Add samples to the Table of Samples to Be Analyzed in LIMS-LSI.
    1. Click the “Projects” button of LIMS-LSI.
    2. Select the project that was logged in.
    3. Click the “Templates” button.
    4. Select “EA for C + N.”
    5. Click “Add” and close LIMS-LSI.
ix. Put samples in cabinets for storage until analyzed.

Appendix B. Step-by-Step Procedure to Generate an Excel Sample Workbook or to Print a Template and a Samples-to-Be-Analyzed List

Excel Sample Workbook

1. Use default worksheet-weighing template and add samples to be analyzed.
2. Fill the appropriate amount in the cells of the rows labeled "Set weight mg."
3. Print first worksheet in workbook.
4. Write the tray ID and the date on both the diskette and paper template.
5. Put them near the balance.

Template

1. Use "Print Template" in LIMS-LSI.
2. Select "appropriate template" for EA and "Delta^{Plus}" for IRMS.
3. Select "New Template" (dialog box informs you how many samples are waiting to be analyzed).
4. Click "OK."
5. Click "Print."
6. Insert diskette to receive sample headings.
7. Click "OK."
8. Write date and day of the week than these samples should be analyzed on both the diskette and paper template.
9. Put them at the balance.
10. Exit LIMS-LSI.

Worksheet 1. Weighing template.

[International reference materials are in bold font.]

		1	2	3	4	5	6	7	8	9	10	11	12
A	Our Lab ID	**G-8396**	**G-8396**	**G-8396**	**G-8396**	**G-8397**	**G-8397**	G-9031	G-9031	G-9032	G-9032	G-9033	G-9033
	Set weight mg	0.75	0.75	0.75	0.75	0.75	0.75	0.8	0.8	0.8	0.8	0.8	0.8
	Actual weight, mg	0.75	0.69	0.73	0.766	0.751	0.764	0.804	0.798	0.799	0.802	0.805	0.803
	Comment	USGS40	USGS40	USGS40	USGS40	USGS41	USGS41						
B	Our Lab ID	G-9034	G-9034	G-9035	G-9035	**G-8396**	**G-8347**	G-1302	G-9037	G-9037	G-9038	G-9038	G-9039
	Set weight mg	0.8	0.8	0.8	0.8	0.75	0.8	0.8	0.8	0.8	0.8	0.8	0.8
	Actual weight, mg	0.801	0.802	0.795	0.803	0.759	0.795	0.801	0.799	0.793	0.804	0.801	0.795
	Comment					USGS40	USGS41						
C	Our Lab ID	G-9039	G-9040	G-9040	G-6741	**G-8396**	**G-8397**	G-9042	G-9042	G-9043	G-9043	G-9044	G-9044
	Set weight mg	0.8	0.8	0.8	0.75	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
	Actual weight, mg	0.804	0.804	0.795	0.766	0.797	0.802	0.8	0.805	0.804	0.797	0.804	0.801
	Comment					USGS40	USGS41						
D	Our Lab ID	G-9045	G-9045	G-9046	G-9046	**G-8396**	**G-8397**	G-9047	G-9048	G-9048	G-9049	G-9049	**G-8396**
	Set weight mg	0.8	0.8	0.75	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
	Actual weight, mg	0.795	0.803	0.766	0.799	0.798	0.796	0.803	0.805	0.805	0.797	0.802	0.796
	Comment					USGS40	USGS41						USGS40
E	Our Lab ID	**G-8397**											
	Set weight mg	0.8											
	Actual weight, mg	0.796											
	Comment	USGS41											

Worksheet 2. Samples to be analyzed.

[International reference materials are in bold font]

Line	Identifier	Port	Comment	Amount	Amt Unit
1	**G-8396**	A1	USGS40	0.75	mg
2	**G-8396**	A2	USGS40	0.769	mg
3	**G-8396**	A3	USGS40	0.763	mg
4	**G-8396**	A4	USGS40	0.766	mg
5	**G-8397**	A5	USGS41	0.751	mg
6	**G-8397**	A6	USGS41	0.764	mg
7	G-9031	A7		0.804	mg
8	G-9031	A8		0.798	mg
9	G-9032	A9		0.799	mg
10	G-9032	A10		0.802	mg
11	G-9033	A11		0.805	mg
12	G-9033	A12		0.803	mg
13	G-9034	B1		0.801	mg
14	G-9034	B2		0.802	mg
15	G-9035	B3		0.795	mg
16	G-9035	B4		0.803	mg
17	**G-8396**	B5	USGS40	0.757	mg
18	**G-8397**	B6	USGS41	0.795	mg
19	G-9036	B7		0.801	mg
20	G-9037	B8		0.799	mg
21	G-9037	B9		0.793	mg
22	G-9038	B10		0.804	mg
23	G-9038	B11		0.801	mg
24	G-9039	B12		0.795	mg
25	G-9039	C1		0.804	mg
26	G-9040	C2		0.804	mg
27	G-9040	C3		0.795	mg
28	G-6741	C4		0.766	mg
29	**G-8396**	C5	USGS40	0.797	mg
30	**G-8397**	C6	USGS41	0.802	mg
31	G-9042	C7		0.8	mg
32	G-9042	C8		0.805	mg
33	G-9043	C9		0.804	mg
34	G-9043	C10		0.797	mg
35	G-9044	C11		0.804	mg
36	G-9044	C12		0.801	mg
37	G-9045	D1		0.795	mg
38	G-9045	D2		0.803	mg
39	G-9046	D3		0.766	mg
40	G-9046	D4		0.799	mg
41	**G-8396**	D5	USGS40	0.798	mg

Line	Identifier	Port	Comment	Amount	Amt Unit
42	**G-8397**	D6	USGS41	0.796	mg
43	G-9047	D7		0.803	mg
44	G-9048	D8		0.805	mg
45	G-9048	D9		0.805	mg
46	G-9049	D10		0.797	mg
47	G-9049	D11		0.802	mg
48	**G-8396**	D12	USGS40	0.796	mg
49	**G-8397**	E1	USGS41	0.796	mg

Appendix C. Step-by-Step Procedure for Weighing and Storing Samples

1. Samples should be homogenized and dried.
2. Insert Template diskette to the computer connected to the microbalance.
3. Condition balance (this step should be done once a day):
   a. Place empty capsule on balance and close door. Wait for reading to stabilize (the "mg" on the display appears). Tare the balance.
   b. Remove and replace capsule and make sure the stabilized weight is 0.000 mg.
   c. Repeat these steps until the balance is stable.
4. Remove capsule from balance, add sample and weigh the filled capsule. Repeat until you have the desired weight (800 ± 10 µg).
5. Fold cup; secure sample in it.
6. Enter sample weight on the template.
7. Repeat steps 4 to 6 for every sample.
8. Notes:
   a. Do not cross-contaminate samples.
   b. Make sure the spatula and sample area are cleaned using Kim wipes.
   c. Always allow balance to stabilize before removing capsule.

Appendix D. Step-by-Step Procedure for Zero Blank Autosampler Operation

1. Before opening lid, make sure the isolation valve (between sample chamber and reaction tube) is closed (arrow toward you), and make sure the He purge isolation valve (on the left) is closed.
2. Open the purge vent on the top of the auto sampler to vent the sample chamber.
3. Release the three fittings holding the lid and open the lid.
4. Place your samples and close the lid. Load 49 samples for the 50-position carousel; leave the first hole empty.
5. Secure the lid with three bolts. Do not tighten one bolt completely. Start all three bolts, and then lightly tighten two at a time, moving around the lid until they are all completely tight.
6. Open He purge isolation valve (screw down), purge the sample chamber for 5 min at He flow rate of 298 mL/min. Make sure the purge vent valve (on top) is open. Press your finger on top of the vent valve for 2 s, then release; you should hear the sound of pressure release.
7. Close the He purge vent valve (screw down), wait for 2 min for gas pressure at sample chamber and He flow rate to stabilize. Close He purge isolation valve.
8. Open isolation valve (arrow toward up), and wait for 2 min for stabilized baseline. Normally when the emission current set at 0.5 mA/0.48 mA, with He carrier flow rate of 90 mL/min, one should see m/z 28 on cup 1 is approximately 10 mV, m/z 29 on cup 2 is approximately 8 to 12 mV, and m/z 30 on cup 3 is approximately 140 mV to 1 V (Appendix J).

Appendix E. Step-by-Step Procedure to Add Sample Information to Sequence Table

1. Insert diskette with “Weighing Template” on it.
2. Open “Sequence File” under “EA configuration.”
3. Open “Weighing Template” in Excel.
4. “Copy” and “Paste” the list of Sample ID, sample weight from template to sequence table.
5. Select appropriate “method” for each sample.
6. Define reference line and blank line.
7. Start.
8. Give Folder Name: Comment.
    a. Select pre: “Date.”
9. Give File Name:
    a. Select pre: “Analyzes #.”
    b. Select post: “Identifier 1.”
    c. Select: “print result.”
10. Click OK.
11. Wait for first sample to be analyzed.

Appendix F Step-by-Step Procedure to Retrieve Data from ISODAT 2.0 for LIMS-LSI and for Data Back-Up

1. For ISODAT 2.0:
    a. Select “Result” in ISODAT 2.0.
    b. Select the result folder you wish to export data from.
    c. Select all the individual analyses you want to retrieve by right clicking selected file.
    d. Select “Reprocess.”
    e. Give a file name.
    f. Add export template previously designed.
    g. Open.
    h. OK. (Reprocessing takes 1-2 min)
    i. Open Excel.
    j. Find the file you reprocessed (E-drive, Finnigan, ISODAT 2.0, Global, User, CONFLO II, Interface, Result: File name).
    k. Open it in Excel.
    1. Save as: “A” drive keep in Lotus format.
    m. Define all reference peaks by adding #1 under column “Is Ref.?”
    n. Now the file can be imported to LIMS-LSI.

2. For Data Back-Up Computer:
    a. Go to Windows explorer in ISODAT 2.0 computer.
    b. Find the drive where the data are (D).
    c. Choose: “Finnigan.”
    d. Choose: “User.”
    e. Choose the inlet system where you have data (Gas Bench or EA).
    f. Choose “Result folder.”
    g. Transfer data to Zip disk or “memory stick.”

Appendix G. Step-by-Step Procedure to Transfer Data to LIMS-LSI, Transfer Data to Data Back-Up Computer, and to Reevaluate Old Data

1. Transfer data to LIMS-LSI:
   a. Start LIMS.
   b. Choose "Import Analysis."
   c. Choose IRMS "Delta Plus."
   d. Click "Import."
   e. Select the file that will be imported.
   f. Select columns containing $\delta^{13}C$ and $\delta^{15}N$ data. Check the boxes to import these two columns.
   g. Click Import. Note: Sample ID, sample weight, Peak Area, Analyzes # are automatically imported to LIMS-LSI.
2. Transfer data to Data Back-Up computer:
   a. Go to Windows Explorer in the Data Back-Up computer.
   b. Find the drive your back-ups are stored.
   c. Choose: "RSIL."
   d. Choose: "Mass. Spec. Analysis Back-Up."
   e. Choose: "P." (Stands for Delta^{Plus} IRMS).
   f. Under that folder create a new one. The name of the folder should be the date range when those analyses were done that you want to back up.
   g. Transfer data to that folder.
   h. Make back-ups every 2 weeks or so, as required.'
3. Reevaluate old data:
   a. Find your samples in the Data Back-Up computer.
   b. Transfer data by a Zip disk or "memory stick" to the computer where a virtual version of ISODAT 2.0 is installed.
   c. Reevaluate your data.

Appendix H. Step-by-Step Procedure to Determine and Apply Correction Factors and Evaluate Data

1. Open “Correction factor and Normalization Equation” in LIMS-LSI.
2. Select the IRMS and isotope, such as “P (Delta Plus) for 15N.”
3. Select “Query.”
4. Double click on the last sample analyzed on that day.
5. Evaluate data of the reference materials.
6. Choose “Normalize with all References.”
7. Accept “Expansion Correction and Additive Correction factors” to samples.
8. Print out correction factor sheet.
9. Report daily reference values and correction factors along with date and range of analysis number to the “EA” binder.
10. Go back to LIMS-LSI main menu by closing open windows.
11. Choose “Print Samples in Progress,”
    a. Open “Sample in Progress.”
    b. Choose appropriate isotope.
    c. Choose appropriate prefix (W for water, N for nitrogen, etc.).
    d. Put in sample ID range from “Samples-to-Be-Analyzed” sheet. Click Print.
12. Review results; determine repeats.
13. Put repeats back to Table of Samples to Be Analyzed.
    a. Go back to LIMS-LSI main menu.
    b. Open “Print Templates.”
    c. Select appropriate template name for the IRMS with which the samples are analyzed.
    d. Find the sample in the “List of Samples.”
    e. Change “Repeats” from 0 to 1.
    f. Close windows and exit LIMS-LSI.

Appendix I. Step-by-Step Procedure to Check Elemental Analyzer for Leaks

1. Close the VENT carrier output using the proper cap (provided).
2. Adjust the He pressure to 150 kPa (regulator is on the front central panel), wait 3 min to equilibrate gas in the system.
3. Close He inlet valve by turning the above regulator counter clockwise.
4. If the gauge needle does not move, there is no leak.
5. If the gauge needle moves, indicating the lost of pressure, there is a leak in the system. The decreasing pressure rate accounts for the degree of leakage.
6. Locate the leak by separating and testing the system segment-by-segment, using He detector.

Appendix J. Daily Checklist

Analyst in charge: _____Date: _____

Weekly:

1. Change working reference gas, O_2 gas, and He gas tanks if the pressure is < 500 psi. Order new tanks for spares; reference gases and oxygen gas are research grade; He carrier is zero grade.
2. Check and clean autosampler carousel.
3. Change reaction tubes (oxidation, reduction) after approximately 600 samples or when it is necessary.
4. Change water trap after approximately 200 samples or when it is necessary.

THESE ITEMS ARE TO BE CHECKED OFF AS YOU CHECK THEM DAILY!

1. Check He, O_2 and reference gas flow. [ ]
2. Check EA reaction-tube temperature (1020 °C and 650 °C). [ ]
3. Change insertion tube (ash collector liner) in the reaction tube. [ ]
4. Check background masses. [ ]

Typical Background at N_2/CO_2 Configuration
Emission Current: 0.54 mA/0.48 mA

Cups	Mass	Intensity (V)	Mass	Intensity (V)	Resistor (Ω)	Capacity (pF)
1	28	0.010		0	3×10^8	680
2	29	0.007	18	1.256	3×10^{10}	5
3	30	0.140	18	6.818	3×10^{11}	2

5. Perform "Ref on/off" method three times to stabilize IRMS. [ ]
6. Check peak center. If it is off default value by 50, do mass calibration. [ ]
7. Do peak jump calibration (from gas N_2 jump to gas CO_2). [ ]
8. Analyze "Ref on/off" method 10 times to stabilize IRMS (std should be < 0.1 ‰). [ ]

ConFlo Preassure Setting N_2, CO_2 (bar)	CO_2 signal (V) Mass 45 on Cup 2	N_2 signal (V) Mass 29 on Cup 2
0.25	0.638	0.173
0.50	1.59	0.450
0.75 Optimal setting CO_2	**2.653**	0.773
1.00	3.921	1.180
1.25	5.315	1.560
1.50 Optimal setting N_2	7.059	**2.074**
1.75	8.778	2.634
2.00	>10.8 (saturated)	3.199

5. Check paper in printer [ ]

Appendix K. Changing the Insertion Tube

The insertion tube needs to be changed before a template is run.

1. Manually close needle valve near the ion source.
2. On the menu under “Spc. Fun.” choose “Std By,” press “enter.” The O_2 and He should be turned off.
3. Unscrew the metal seals under the sample carousel to access the reaction tube.
4. Using the heat protective glove and the wire tool remove the insertion tube from the reaction tube and place in the metal can. Be aware that the tube will be very hot!
5. Insert clean tube from the drawer that has 0.5 cm of quartz wool packed on the bottom and tap it down with the wire tool.
6. Replace the carousel and tighten the valves with a wrench.
7. Turn the O_2 and He back on by pressing “enter” and return the screen to the temperature readout by pressing “Spc. Fun.”
8. Open the needle valve.

Appendix L. Changing the Water Trap

Change the water trap after every 200 samples.

1. Retrieve quartz turnings, quartz wool, magnesium perchlorate, and a clean water trap from Delta^{Plus} supplies.
2. Under a hood, pack one end of the clean water trap with 1 cm of quartz wool.
3. On a piece of wax paper, create a mix of about 70 % magnesium perchlorate and 30 % quartz turnings, pack the mix into the water trap tube, leaving 1 cm of empty space.
4. Pack the open end of the water trap with quartz wool.
5. Unscrew the red plastic ends from the used water trap and replace it with the clean water trap, be sure to replace the rubber O-rings on the ends of the clean water trap and tighten the red ends.
6. Insert the plug into the vent and watch for any drop in pressure to check for a leak in the system. After 5 minutes if everything looks acceptable, replace the vent line.
7. Under the "Spc. Fun." Menu of the model 2500 EA, choose "CNT" (counter) and zero "D," to set back the water-trap counter.
8. Clean the water trap by pushing all the mixture out of the tube into the trash can and use a clean cotton swab to wipe out the inside of the tube. Rinse the tube with DIW, and dry it in the oven. Return the now clean tube to the water trap bag in the drawer.

Appendix M. Changing the Reaction Tubes

The reaction tubes should be changed about every 500 to 600 samples. [The new reaction tubes will need to equilibrate over night before they can provide accurate results; thus, change the reaction tubes at the end of the day.]

1. Manually close the needle valve near the ion source.
2. Turn the model 2500 EA to “Std By” with both gases off.
3. Lower the oxidation tube temperature to less than 900 °C; leave the reduction tube temperature as it is.
4. Retrieve the reaction tubes from the cabinet.
5. Insert the insertion tube into the top of the oxidation tube; there will already be a plastic tube in the reaction tube that should be thrown away.
6. Place black O-rings on the top of the reaction tubes. Make sure that all O-ring connections are clean and free of any dust.
7. Loosen the top two valves and then remove the bottom valve. Go back and completely remove the top valve. Very hot! Use oven gloves!
8. Remove quartz reaction tubes and place them in metal can. Place clean reaction tubes in column. Make sure the O-rings are clean, then replace the top.
9. On the bottom , put on the metal pieces with metal O-rings first and then add the clean rubber O-rings. Raise the bottom portion and screw together. Then tighten the connections with wrenches, put the metal supports back in place.
10. Turn the gases back on, then go to “CNT” in the menu under “Spc. Fun.” Set to zero the counter of the oxidation reaction tube, “B,” as well as that of the reduction reaction tube “C.”
11. Insert the plug into the vent and close the He supply. Watch for any drops in the pressure to check for a leak in the system. After 3 minutes if everything looks OK, open the He supply and replace the vent line.
12. Open the needle valve.

Appendix N. Step-by-Step Procedure to Report Data

1. Open “Store Samples in Progress” in LIMS-LSI.
2. Choose appropriate isotope.
3. Choose sample ID range from “Sample in Progress” print out.
4. Store data.
5. Go back to LIMS-LSI main menu.
6. Open “Project” and find the appropriate project in the list.
7. Select “Print Report” and check whether the project report contains all the results. If not, search for the missing results in the database.
8. Select “Results,” transfer data in Excel format or (and) text format to a diskette, and report data to customer through e-mail.
9. Click “Print Report” to print a project report and put it in the “Correspondence” binder along with all other documents in the “Samples In Progress” binder that are related to this project.
10. If the samples were submitted through the NWQL, click “NWQL Export” and transfer data in NWQL format to a disc; report these data to the NWQL by e-mail. Print a copy of the NWQL export file and save in the “Correspondence” binder with the project report printed in the step above.