



Determination of the $\delta(^{15}\text{N}/^{14}\text{N})$ of Ammonium (NH_4^+) in Water: RSIL Lab Code 2898

Chapter 15 of
Book 10, Methods of the Reston Stable Isotope
Laboratory
Section C, Stable Isotope-Ratio Methods

Techniques and Methods 10–C15

U.S. Department of the Interior
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By Janet E. Hannon and John Karl Böhlke

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Edited by Kinga Révész and Tyler B. Coplen

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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.



Pierre D. Glynn
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Conversion Factors

Multiply	By	To Obtain
Length		
micrometer (μm)	0.00003937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
centimeter (cm)	0.3937	inch (in.)
meter (m)	3.281	foot (ft)
Volume		
microliter (μL)	0.0610210^{-3}	cubic inch (in^3)
milliliter (mL)	0.06102	cubic inch (in^3)
cubic centimeter (cm^3)	0.06102	cubic inch (in^3)
liter (L)	61.02	cubic inch (in^3)
Mass		
nanogram (ng) = 10^{-3} μg	3.527×10^{-11}	ounce (oz)
microgram (μg) = 10^{-3} mg	3.527×10^{-8}	ounce (oz)
milligram (mg) = 10^{-3} g	3.527×10^{-5}	ounce (oz)
gram (g) = 10^{-3} kg	3.527×10^{-2}	ounce (oz)
kilogram = 10^3 g; 10^6 mg; 10^9 μg ; 10^{12} ng	2.205	pound (lb)
Density		
gram per cubic centimeter (g/cm^3)	0.5780	ounce per cubic inch (oz/in^3)
Temperature		
Celsius ($^{\circ}\text{C}$)	$^{\circ}\text{F} = 9/5 (^{\circ}\text{C}) + 32$	Fahrenheit ($^{\circ}\text{F}$)
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 Standards Institute
ASR	analytical services request
CF-IRMS	continuous flow isotope-ratio mass spectrometer
DIW	deionized water
EA	elemental analyzer

GC	gas chromatograph
GF	glass fiber
HDPE	high-density polyethylene
IAEA	International Atomic Energy Agency
IRMS	isotope-ratio mass spectrometer
LIMS-LSI	Laboratory Information Management System for Light Stable Isotopes
mol/L	mol per liter
mmol/L	millimol per liter
mg/L	milligram per liter
<i>m/z</i>	mass-to-charge ratio
MSDS	Material Safety Data Sheets
NBS	National Bureau of Standards
NFPA	National Fire Protection Association
NWQL	National Water-Quality Laboratory (USGS)
PCM	polycarbonate membrane
per mill, ‰	parts per thousand, 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

Symbols

Ω	ohm
<	less than
>	greater than
\leq	less than or equal to
\geq	greater than or equal to

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Summary of Procedure

The purpose of the technique described by Reston Stable Isotope Laboratory (RSIL) lab code 2898 is to determine the N isotopic composition, $\delta(^{15}\text{N}/^{14}\text{N})$, abbreviated as $\delta^{15}\text{N}$, of ammonium (NH_4^+) in water (freshwater and saline water). The procedure involves converting dissolved NH_4^+ into NH_3 gas by raising the pH of the sample to above 9 with MgO and subsequently trapping the gas quantitatively as $(\text{NH}_4)_2\text{SO}_4$ on a glass fiber (GF) filter. The GF filter is saturated with NaHSO_4 and pressure sealed between two gas-permeable polypropylene filters. The GF filter “sandwich” floats on the surface of the water sample in a closed bottle. NH_3 diffuses from the water through the polypropylene filter and reacts with NaHSO_4 , forming $(\text{NH}_4)_2\text{SO}_4$ on the GF filter. The GF filter containing $(\text{NH}_4)_2\text{SO}_4$ is dried and then combusted with a Carlo Erba NC 2500 elemental analyzer (EA), which is used to convert total nitrogen in a solid sample into N_2 gas. The EA is connected to a continuous-flow isotope-ratio mass spectrometer (CF-IRMS), which determines the relative difference in ratios of the amounts of the stable isotopes of nitrogen (^{15}N and ^{14}N) of the product N_2 gas and a reference N_2 gas. The filters containing the samples are compressed 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 O_2 gas. To remove S-O gases produced from the NaHSO_4 , a plug of Ag-coated Cu wool is inserted at the bottom of the reaction tube. Combustion products are transported by a He carrier through a reduction furnace to remove excess O_2 , to convert all nitrogen oxides to N_2 , and to remove any remaining S-O gases. The gases then pass through a drying tube to remove water. The gas-phase products, mainly N_2 and a small amount of background CO_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 to introduce not only sample into the IRMS but also N_2 reference gas and He for sample dilution. The flash combustion is quantitative, so no isotopic fractionation is involved. The IRMS is a Finnigan Delta V CF-IRMS with 10 cups and is capable of detecting ion beams with mass/charge (m/z) 28, 29, 30. The ion beams from N_2 are as follows: m/z 28 = $^{14}\text{N}^{14}\text{N}$, m/z 29 = $^{14}\text{N}^{15}\text{N}$, and m/z 30 = $^{15}\text{N}^{15}\text{N}$. The ion beam with m/z 30 also represents $^{14}\text{N}^{16}\text{O}$, which may indicate contamination or incomplete reduction.

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 number 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 that of an international measurement standard. This relative difference is designated $\delta^i\text{E}$, which is a shortened form of $\delta(^i\text{E}/^j\text{E})$ or $\delta(^i\text{E})$ and is defined according to equation 1:

$$\delta^i E = \delta(^i E) = \delta(^i E, ^j E) = \frac{n_B(^i E)/n_B(^j E) - n_{\text{std}}(^i E)/n_{\text{std}}(^j E)}{n_{\text{std}}(^i E)/n_{\text{std}}(^j E)} \quad (1)$$

where i and j are isotopes of element E and $n_B(^i E)/n_B(^j E)$ and $n_{\text{std}}(^i E)/n_{\text{std}}(^j E)$ are the ratios of the isotope amounts in the unknown B and the international measurement standard (std). A positive $\delta^i E$ value indicates that the unknown is more enriched in the heavy isotope ($^i E$) than the international measurement standard. A negative $\delta^i E$ value indicates that the unknown is depleted in the heavy isotope relative to the international measurement standard. Values of $\delta^i E$ commonly are reported in parts per hundred (% or percent), parts per thousand (‰ or per mill), parts per ten thousand, or parts per million. For stable nitrogen isotope–amount ratios, $\delta^{15}\text{N}$ is defined as follows:

$$\delta^{15}\text{N} = \delta(^{15}\text{N}) = \delta(^{15}\text{N}, ^{14}\text{N}) = \frac{n_B(^{15}\text{N})/n_B(^{14}\text{N}) - n_{\text{std}}(^{15}\text{N})/n_{\text{std}}(^{14}\text{N})}{n_{\text{std}}(^{15}\text{N})/n_{\text{std}}(^{14}\text{N})} \quad (2)$$

The international measurement standard for relative nitrogen isotope–ratio measurements ($\delta^{15}\text{N}$) is atmospheric nitrogen gas (N_2), which is widespread and homogeneous and, by convention, has a $\delta^{15}\text{N}$ value of 0 ‰ (Mariotti, 1983). By interspersing isotopic reference materials with accepted δ values among unknown samples, the δ values can be determined on a normalized $\delta^{15}\text{N}$ scale. The $\delta^{15}\text{N}$ measurements are made with a CF-IRMS, which alternately measures the relative isotope–amount ratios of the sample N_2 gas and one or more injections of the “working reference” N_2 gas.

Analyses of dissolved ammonium samples normally are calibrated by analyzing solutions containing $(\text{NH}_4)_2\text{SO}_4$ with known $\delta^{15}\text{N}$ values ranging from approximately 0 ‰ to +54 ‰, which covers the range of $\delta^{15}\text{N}$ values of most natural samples in the environment (Coplen and others, 2002). A single (nonreplicated) analysis requires a minimum of 2 to 3 μmol of sample as N . Values of $\delta^{15}\text{N}$ are reported in per mill at the RSIL. Replicated analyses have typical reproducibilities of approximately ± 0.4 ‰. Artificially enriched samples with wider ranges of isotopic compositions can be analyzed by using alternative reference materials with $\delta^{15}\text{N}$ values ranging from approximately -30 ‰ to +5000 ‰, with typical reproducibilities increasing to approximately ± 100 ‰ at the upper end of that range.

Reference Materials and Documentation

Reference Material Used, Storage Requirements, and Shelf Life

In this method, two pure ammonium sulfate $(\text{NH}_4)_2\text{SO}_4$ reagents normally are used as in-house reference materials when natural samples are analyzed. These are N-8 with $\delta^{15}\text{N} = +0.52$ ‰ and N-6 with $\delta^{15}\text{N} = +53.7$ ‰, when normalized to international reference materials IAEA-N1 with $\delta^{15}\text{N} = +0.43$ ‰ and USGS26 with $\delta^{15}\text{N} = +53.7$ ‰ (Böhlke and Coplen, 1995; Qi and others, 2003). When artificially enriched or depleted samples are analyzed, other in-house reference materials may be used. These include N-5 with $\delta^{15}\text{N} = -30.18$ ‰ and N-29 with $\delta^{15}\text{N} = +5230$ ‰ when normalized to international reference materials USGS25 with $\delta^{15}\text{N} = -30.18$ ‰ and IAEA-311 with $\delta^{15}\text{N} = +4731$ ‰. Detailed preparation and calibration procedures are given in Böhlke and others (1993), Böhlke and Coplen (1995), and Qi and others (2003).

The in-house and the international reference materials are stored in the RSIL in glass bottles. 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: N-5, N-6, N-8, and N-29 as in-house reference materials and N-1 (IAEA-N-1), N-5 (USGS25), N-6 (USGS26) N-19 (IAEA-311) as international reference materials.

Labware, Instrumentation, and Reagents

Labware, instrumentation, and reagents for sample preparation include the following: 5 mmol/L solutions of two $(\text{NH}_4)_2\text{SO}_4$ isotopic reference materials; 3 mol/L solution of reagent NaHSO_4 , MgO , and NaCl (both precombusted for 4 h at 475 °C); GF filters, grade C (GF/C), cut to 1.0 cm diameter; polypropylene filters, 2.5 cm diameter; gloves; blunt-tipped forceps; micro spatulas; padded working surface; 20 mL scintillation vials (precombusted for 4 h at 475 °C); 60 mL or 250 mL acid-washed (with 10 % HCl) high-density polyethylene (HDPE) bottles with tight-fitting caps; pH meter and electrode; pH buffer solutions; stirring plate; stirring bars; pipette accurate to between 20 and 300 μL ; pipette tips; rotating, heated hybridization oven; vacuum oven; desiccator containing a bowl of concentrated H_2SO_4 in the bottom; tin capsules; and a microbalance capable of measuring samples with 0.001-mg precision.

The isotopic analytical apparatus consists of four different components: (1) EA, (2) ConFlo interface, (3) IRMS, and (4) computer software (Révész and Qi, 2006).

The EA is a Carlo Erba Instruments NC 2500 system (fig. 1), and it is equipped with a Costech “zero blank” autosampler that holds 49 samples enclosed in tin capsules. 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, then through a plug of Ag-coated Cu wool at the bottom of the reaction tube. The gas then flows through a reduction agent (Cu) in another reaction tube kept at 650 °C where any nitrogen oxides are converted to elemental nitrogen, any excess oxygen is consumed, and any remaining S-O gases are removed. At the outlet of the second reaction tube, the gas mixture (N_2 , background 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 CO_2 gas pass separately through the thermal conductivity detector, through the ConFlo II open split, and into the IRMS.

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 the 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 V 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 a Faraday cup collector. The ion-beam intensities are measured with electrometers. This IRMS has 10 collectors and is capable of simultaneously measuring m/z 28, 29, and 30. The resistor–capacitor combination on the electrometers used to measure

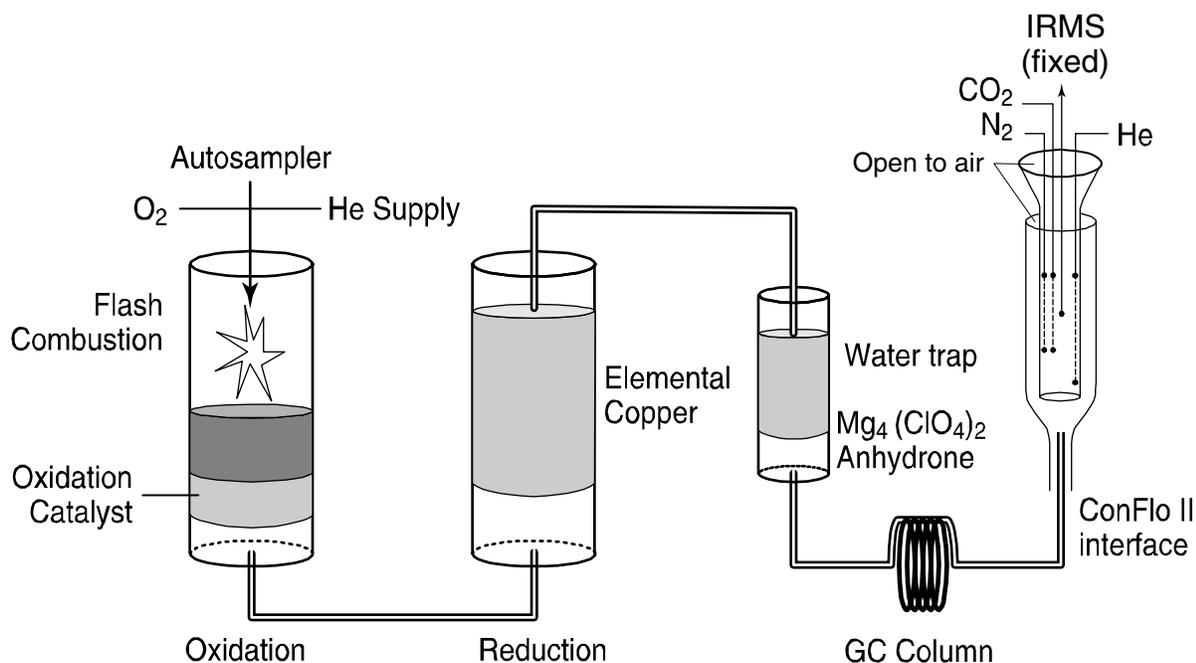


Figure 1. Diagram of the Carlo Erba Instruments NC 2500 E (CE NC 2500) Elemental Analyzer (used with permission from Thermo Electron Co.) connected to Finnigan ConFlo II Open Split.

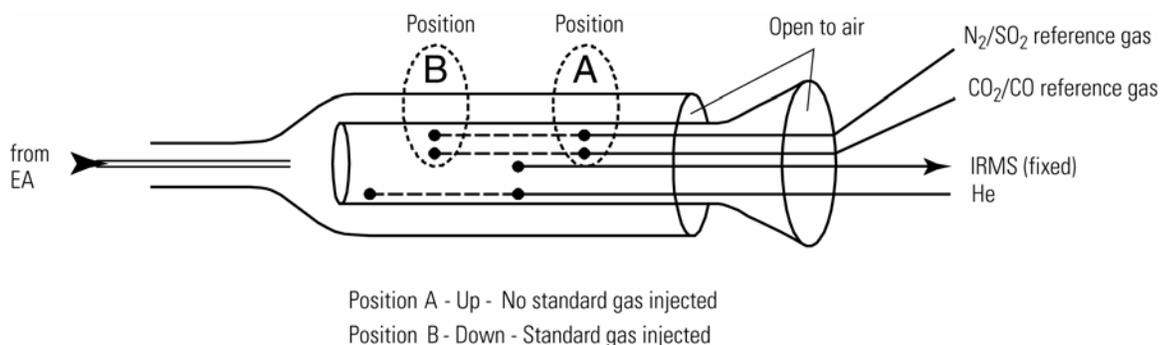


Figure 2. Diagram of a Finnigan ConFlo II Open Split (used with permission from Thermo Electron Co.)

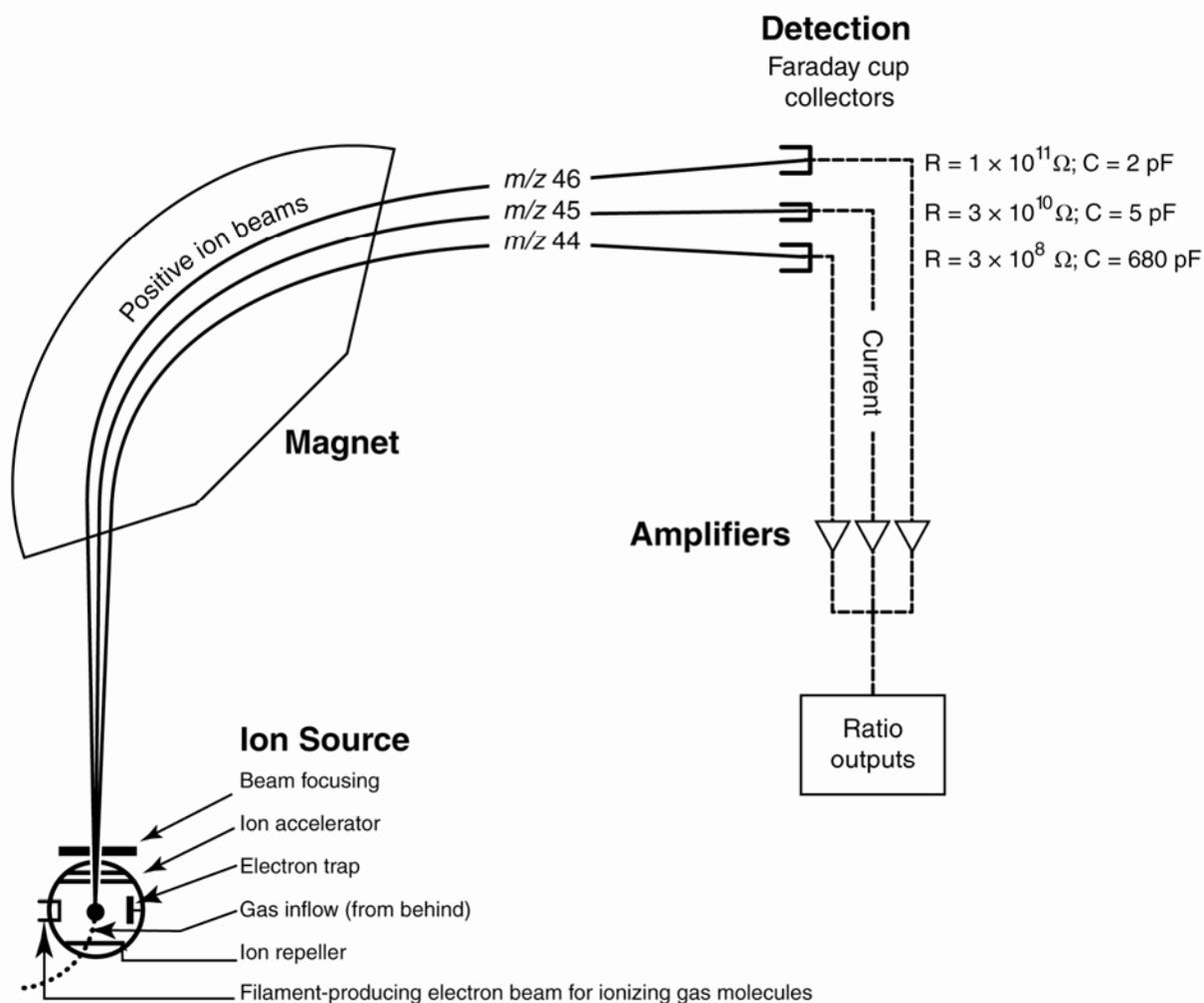


Figure 3. Schematic of a continuous flow isotope-ratio mass spectrometer (CF-IRMS) (modified from Clark and Fritz, 1997).

these respective ion beams are as follows: $3 \times 10^8 \Omega$ on cup 1, $3 \times 10^{10} \Omega$ on cup 2, $1 \times 10^{11} \Omega$ on cup 3. The Finnigan ISODAT 2.5 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 δ values.

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

Sample Container, Preservation, and Handling Requirements

Each sample is collected in an HDPE bottle of appropriate size for concentration of sample with Polyseal cap. The maximum sample size is 1 L. The bottles are field rinsed, and the sample is filtered through a 0.45- μm filter directly into the bottle, leaving a small headspace. Ammonium-free concentrated H_2SO_4 (typically from sealed glass ampoules) is added to the sample to achieve a pH value of less than or

equal to 2. Containers are labeled with isotopes whose relative isotope-amount ratios are to be determined and with the respective laboratory code or schedule number. The label must indicate that H₂SO₄ has been added. Samples may be shipped chilled or at ambient temperature. The submitter must provide concentration of ammonia in sample to RSIL with the sample shipment. Concentrations provided by the National Water Quality Laboratory (NWQL) analysis (for example, Lab Code 3116) are satisfactory. The minimum quantity of ammonium in the sample must be at least 0.2 mg as N, and the minimum concentration of ammonium is 0.2 mg/L as N. Field IDs on the bottle must match the information provided with concentrations.

Sample Preparation

Sample preparation involves logging in samples to the LIMS-LSI, processing samples to produce (NH₄)₂SO₄, creating a sample work-order sheet, and weighing samples into capsules.

The logging-in procedure involves entering sample information into LIMS-LSI in batches 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 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 for sample log-in are given in Appendix A.

The following procedure is modified from that of Holmes and others (1998). An Excel spreadsheet is created to calculate the amount of each sample needed to contain 2 to 3 μmol NH₄⁺, based on the NH₄⁺ concentrations provided by the submitter. Sample pH values are measured to ensure preservation to less than a value of 2.5. For samples that do not meet this criterion, the pH is adjusted with concentrated H₂SO₄, and the spreadsheet is annotated accordingly. Aliquots containing 2 or 3 μmol of NH₄⁺ are transferred to 60 mL acid-washed HDPE bottles labeled with the Lab ID. Ammonium isotopic reference solutions containing 2 or 3 μmol NH₄⁺ [200 or 300 μL of 5 mmol/L (NH₄)₂SO₄ adjusted to pH 2 with H₂SO₄] are pipetted into the same-sized bottles. Deionized water (DIW) is added to each bottle if necessary to bring the total liquid volume to 30 mL. The pH in each bottle is checked again. If it is lower than 2, it is adjusted to between 2.0 and 2.5 by adding LiOH. Aliquots of NaCl are added to each bottle to increase the total salinity to approximately 35 g/L. This decreases the solubility of the NH₃ and increases the osmotic pressure of the solution, which maintains the integrity of the diffusion sandwiches.

If the required amount of a sample exceeds 30 mL per aliquot, then the calculated sample amount for an analysis is measured into a clean container, frozen, and placed in a freeze dryer to concentrate the sample below 30 mL. Alternatively, the amounts of samples and reference solutions may be increased and loaded into correspondingly larger bottles (for example, 200 mL total volume in a 250 mL bottle).

Diffusion packets are constructed using gloves and a clean working surface. The packets consist of a 1-cm-diameter GF/C filter containing 20 μL of 3 mol/L NaHSO₄ sandwiched between two polypropylene membrane filters. The polypropylene filters are sealed together using the smoothed end of a stainless steel tube pressed down on the filters and rotated slightly to form a circular compression around the enclosed filter. The filter packets can be assembled several days ahead of use and stored in a tightly capped wide-mouth bottle. A diffusion packet is placed in each bottle containing a sample or reference solution, MgO is added (0.35–1.0 g) to the bottle to increase pH and convert NH₄⁺ to NH₃, and the bottle is capped immediately to prevent loss of gaseous NH₃. One bottle is prepared as a method blank with the same volume DIW, headspace, reagents, and reaction conditions as the samples and references. The batch of

bottles containing diffusion packets is placed in a rotating hybridization/incubation oven at 45 °C for 72 h (or longer, as appropriate for larger sample volumes, for example, 10 days for 250 mL bottles). The diffusion packets are then removed from the bottles and placed in individual prebaked glass scintillation vials labeled with the Lab ID. The vials containing diffusion packets are dried for 2 h in a vacuum oven at 40 °C.

The vials with samples are placed in a glass desiccator containing a bowl of concentrated H₂SO₄ in the base of the desiccator. After 24 h of drying in the desiccator, vials are removed and capped; they may be stored for up to 2 weeks. For IRMS analysis of δ¹⁵N, the GF/C filters are removed from the polypropylene sandwiches using gloves, loaded into tin foil capsules, and analyzed immediately by EA-IRMS. The detailed procedure is given in Appendix B.

An Excel sample workbook is generated. Step-by-step procedures and examples are given in Appendix C.

For pure solid reference materials, sample weighing includes conditioning the balance, weighing sufficient reference sample to yield 30 to 40 μg of N into capsules, recording weight on the template, and closing capsules by folding them. Filters from the diffusion procedure are not weighed, but the Lab ID is recorded in the template along with a comment that this is a filter sample. Step-by-step procedure is given in Appendix D.

Performing the Analysis

All samples are prepared and analyzed in batches, and all samples are analyzed in replicate on different days to minimize bias associated with the day-to-day uncertainties in the measured values of the reference materials.

Each batch of samples contains one diffusion packet that has been reacted identically to the samples and references, but with no sample or reference added, to determine if a blank is produced in the reaction. This blank sample has an LIMS-LSI number N-13273. Three aliquots each of two different isotopic reference materials (N-6 and N-8) are analyzed as pure solid salts at the beginning of a batch. One set is also analyzed at the end of the batch. Each batch includes three to four aliquots of each in-house isotopic reference material that have been dissolved in water, acidified, and collected on GF filters in an identical method as the samples. Pairs of these two reference materials are interspersed among the samples. No fewer than one reference material set is analyzed for every eight unknowns analyzed.

The procedure involves loading the autosampler carousel with capsules in sequence according to the “Template” (Appendix E), loading a new sample heading (including sample weights) into the “Sequence Table” in ISODAT, choosing the appropriate “Method,” and starting sequence acquisition (Appendix F). After the analyses are completed, the data are exported (Appendix G) to a ZIP disk or “memory stick” from the ISODAT database and imported (Appendix H) to the LIMS-LSI and transferred to the Data Backup computer. Final daily correction factors are determined with LIMS-LSI (Appendix I), based on the daily analyses of reference materials using equations 3 and 4, which are presented with numerical values:

$$+0.52 \text{ ‰} = m \times \delta_{\text{N-8} - \text{workingrefgas}}^{15}\text{N} + b \quad (3)$$

$$+53.70 \text{ ‰} = m \times \delta_{\text{N-6} - \text{workingrefgas}}^{15}\text{N} + b \quad (4)$$

The values +0.52 ‰ and +53.7 ‰ are the calibrated δ¹⁵N values of the two in-house reference materials N-8 and N-6, respectively. The δ¹⁵N values on the right side of equations 3 and 4 are the mean daily values of the respective in-house reference materials, prepared and treated as aqueous samples, relative to the working reference gas; *b* is the additive correction factor; and *m* is the expansion coefficient correction factor.

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 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 have organic compounds (especially amines) that are volatile or that may affect the diffusion process. Concentrations of NH_4^+ less than 0.2 mg/L as N sometimes do not produce results that are reproducible. Samples that are not acidified in the field may be altered during storage.

Interferences

No interferences, other than the ones described above as “Problematic Samples,” are known.

Troubleshooting and Bench Notes

Some potential problems with the sample preparation include (1) incomplete diffusion of NH_3 out of the water and into the filter sandwiches, (2) leakage of water into the filter sandwiches, and (3) contamination by organic N. These problems may be evident as anomalous yields of N_2 for individual samples. They also may be evident as poor reproducibility of measured $\delta^{15}\text{N}$ values of reference materials in a given batch or of sample aliquots in different batches. Analyses are rejected when yields or $\delta^{15}\text{N}$ values are anomalous or not reproducible.

Some problems with the combustion and mass spectrometry of prepared samples include (1) incomplete combustion; (2) leakage in the system, particularly in the EA, and (3) insufficient linearity of the IRMS (Révész and Qi, 2006).

The fact that the melting points of the compounds being combusted may be 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 setup parameters of the RSIL EA are as follows: 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. The 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. Accumulation of solid residues also may be problematic. Residues consist in part of SnO , which serves as a catalyst in the reaction, and glass from the filters containing samples. Accumulation of excess residues can interfere with the flow rate, and possibly the reaction conditions, and occasionally causes systematic changes in the measured $\delta^{15}\text{N}$ values during a batch of samples. By frequently analyzing reference materials, this shift can be corrected. In addition, the residues are removed after each batch of samples (typically 30–50 samples). The leak-checking procedure is given in Appendix J. 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 40 to 50 mV with typical IRMS settings. (See “Daily Checklist” in Appendix K.)

The IRMS is focused electronically. See the manufacturer’s manual for instructions. Emission current is also critical. The higher the emission current is, the higher the signal is but the poorer the linearity will be. 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 measuring m/z 28, 29, and 30 are as follows: $3 \times 10^8 \Omega$ for cup 1, $3 \times 10^{10} \Omega$ for cup 2, and $1 \times 10^{11} \Omega$ for cup 3. For normal materials (not enriched in ^{15}N), m/z 30 commonly represents $^{14}N^{16}O$, which may be an indication of contamination or incomplete combustion.

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 $\pm 0.1 \%$ or better for $\delta^{15}N$. The “Ratio Linearity” test must give 0.12 $\%/V$ or better linearity. An external precision check should be performed as needed, approximately every week or so; samples of the same amount need to be analyzed within ± 0.010 mg. The $1 - \sigma$ standard deviation must be $\pm 0.2 \%$ or better for $n = 10$.

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, including date of oil changes, problems, and repairs, are listed in a pump maintenance database. Maintenance of the EA includes (1) changing the insertion tube after each batch run (Appendix L), (2) changing the water trap approximately every 200 samples (Appendix M), (3) changing reaction tubes when necessary between 200 to 250 analyses (Appendix N), and (4) cleaning the sample carousel every week. The IRMS also requires a daily check (Appendix K). A logbook is kept for each IRMS, where notes of maintenance checks, history of normal settings, problems, and repairs are listed.

Maintenance of ISODAT includes importing (transferring) results from the ISODAT database to the LIMS-LSI computer hard disk and the Data Backup 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 Backup computer. Paper reports are kept for a few weeks. Analytical results from the IRMS are transmitted to the LIMS-LSI and are 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 Isotope (LIMS-LSI)

In the RSIL, the LIMS-LSI (Coplen, 2000) is used for data acquisition, processing, and evaluation. This system is a database program capable of (1) storing information about samples, (2) storing the results of mass spectrometric δ values of samples after importing from the ISODAT database, (3) calculating analytical results using standardized algorithms stored in a database, (4) normalizing δ 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

The in-house ammonium isotopic reference materials prepared and analyzed as aqueous samples with each batch of unknowns serve as QC samples. The preparation and analytical cost of these isotopic in-house reference materials is the responsibility of RSIL. Both RSIL and NWQL monitor these results.

After each batch of analyses, 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 using the values from isotopic reference materials collected on glass fiber filters, 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 and determines which samples need to be reanalyzed to achieve acceptable results ($\pm 0.4\%$, $1 - \sigma$).

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:

- If the standard deviation is less than $\pm 0.4\%$, use mean delta.
- If there are three or more analyses, delete the outlier and recalculate.
- If standard deviation of this recalculation is 0.4% , use mean from this recalculation.
- 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, or if the N yields from the samples are significantly different from the expected yields based on reported concentrations, the analyst contacts the supervisor. The troubleshooting 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 reports 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 about problematic results.

Data Management and Records

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

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 are available using the URL <http://www.ilpi.com/msds/>. Manufacturers provide links to MSDSs of most chemicals.

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. Because the instruments require a third wire protective grounding conductor, three-to-two wire adapters are unsafe for them.

Chemical Hazards

Potentially hazardous chemicals used in the preparation of the samples include H₂SO₄, NaHSO₄, and LiOH in various concentrations. The hazardous chemicals used in the EA combustion process are Cr₂O₃ and Co₃O₄/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 USGS *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 $\text{Co}_3\text{O}_4/\text{Ag}$ must be collected in a closed container and given to the safety, health, and environment officer for disposal. Solutions containing MgO in water must be neutralized before disposal in the sink.

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Summary of Product Components

Component	Description	Last Revised In Publication Version	Date of Last Revision

List of Revisions (latest version first):

References Cited

- Böhlke, J.K., and Coplen, T. B., 1995, Interlaboratory comparison of reference materials for nitrogen-isotope-ratio measurements, reference and intercomparison materials for stable isotopes of light elements, IAEA TECDOC 825: Vienna, Austria, International Atomic Energy Agency, p. 51–66. http://www-pub.iaea.org/MTCD/publications/PDF/te_825_prn.pdf.
- Böhlke, J.K., Gwinn, C.J., and Coplen, T.B., 1993, New reference materials for nitrogen-isotope-ratio measurements: *Geostandards Newsletter*, v. 17, p. 159–164.
- Brenna, J.T., Corso, T.N., Tobias, H.J., and Caimi, R.J., 1997, High-precision continuous-flow isotope ratio mass spectrometry: *Mass Spectrometry Reviews*, v. 16, p. 227–258.
- Clark, Ian, and Fritz, Peter, 1997, *Environmental Isotopes in Hydrogeology*: Boca Raton, Fla., Lewis Publishers, 328 p.
- Coplen, T.B., 2000, A guide for the laboratory information management system (LIMS) for light stable isotopes—Version 7 and 8: U. S. Geological Survey Open-File Report 00-345, 121 p.
- Coplen, T.B., Hopple, J.A., Böhlke, J.K., Peiser, H.S., Rieder, S.E., Krouse, H.R., Rosman, K.J.R., Ding, T., Vocke, R.D., Jr., Révész, K.M., Lamberty, A., Taylor, P.D.P., and De Bièvre, P., 2002, Compilation of minimum and maximum isotope ratios of selected elements in naturally occurring terrestrial materials and reagents: U.S. Geological Survey Water-Resources Investigations Report 01-4222, 98 p.
- Holmes, R.M., McClelland, J.W., Sigman, D.M., Fry, B., and Peterson, B.J., 1998, Measuring $^{15}\text{NH}_4^+$ in marine, estuarine, and fresh waters: an adaptation of the ammonia diffusion method for samples with low ammonium concentrations: *Marine Chemistry*, v. 60, p. 235–243.
- Mariotti, A., 1983, Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements: *Nature*, v. 303, p. 685–687.
- Révész, K., and Qi, H., 2006, Determination of the $\delta(^{15}\text{N}/^{14}\text{N})$ of total nitrogen in solids, chap. C11 of Révész, Kinga, and Coplen, Tyler B., eds., RSIL lab code 2893, *Methods of the Reston stable isotope laboratory*: Reston, Virginia, U.S. Geological Survey, 31 p.
- Qi, H., Coplen, T.B., Geilmann, H., Brand, W.A., and Böhlke, J.K., 2003, Two new reference materials for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements and a new value for the $\delta^{13}\text{C}$ of NBS 22 oil: *Rapid Communications in Mass Spectrometry*, v. 17, p. 2483–2487.
- U.S. Geological Survey, 2002, USGS Handbook 445-3-H, *Safety and Environmental Health Handbook*, 435 p.

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.
 - b. Assign NWQL #s based on the Julian date of the Monday following the submission date. Check note on the bulletin board for "Next NWQL #." Write NWQL #s on ASR forms, then update "Next NWQL #" on the 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)].
 - l. 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. Box 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. Store samples 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 isotopes@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. Print out one project reports and one container label.
 - iv. Put a label on a sample bottle and crosscheck Field IDs between bottles and Excel Worksheet forms.
 - v. Enter G-# range into the appropriate template.
 - vi. Punch holes in original Excel Worksheet and all the information of the project, and put in the Samples-in-Progress binder.
 - vii. Store samples until analyzed.

Appendix B. Step-by-Step Procedure to Convert NH_4^+ in Water to NH_3 Gas, Then to $(\text{NH}_4)_2\text{SO}_4$

- Using an Excel spreadsheet, calculate the volume of sample needed to contain between 0.14 and 0.21 mg NH_4^+ as N.
 - For samples with concentrations of less than 0.9 mg/L as N, the volume containing 2 μmol will be greater than 30 mL and must be freeze dried first to reduce the volume. Check the pH of the sample to ensure it is approximately 2, measure the amount needed, record the actual amount used, freeze sample, then freeze dry. Remove from freeze drier when volume is less than 30 mL, and add DIW to bring the volume to 30 mL. Alternatively, adjust the volumes of all samples and reference solutions in a given batch to some larger amount (for example, 200 mL of solution in a 250 mL bottle).
 - Samples with concentrations from 0.9 to 1.4 mg/L as N should be measured to contain 2 μmol NH_4^+ . Record the sample amount used and add DIW to bring the final volume to 30 mL.
 - Samples with a concentration greater than 1.4 mg/L as N should be measured to contain 3 μmol NH_4^+ . Record the sample amount used and add DIW to bring the final volume to 30 mL.
- Transfer sample aliquots containing 2 to 3 μmol of NH_4^+ to 60 mL or 250 mL acid-washed HDPE bottles. Record volume used. Dilute each bottle if needed, with DIW to a constant total volume of 30 mL or 200 mL. If the pH is less than 2, adjust it to between 2.0 and 2.5 with LiOH.
- Pipette isotopic in-house reference solutions containing 2 or 3 μmol NH_4^+ to match the concentrations of the samples [200 or 300 μL of the standard solution – 5 mmol/L $(\text{NH}_4)_2\text{SO}_4$]. Dilute each reference with DIW to the same constant total volume as the samples and adjust the pH to 2 with H_2SO_4 . Prepare one bottle with either 30 mL or 200 mL DIW at pH 2 for use as a method blank.
- Standardize the salinities of each sample, reference or blank to 35 g/L by adding the appropriate amount of NaCl (precombusted to 475 °C for 4–5 h).
- To make diffusion packets, use a piece of aluminum foil folded over to form 4 to 6 layers. Use gloves and completely clean foil and two pair of forceps with methanol and wipes. The diffusion packets consist of a 2.5-cm diameter GF/C filter, precombusted to 475 °C for 4 to 5 h and cut to 1 cm in diameter. Pipette 20 μL of 3 mol/L NaHSO_4 onto the GF filter. Sandwich the GF/C filter between two 2.5-cm polypropylene membrane filters. Seal the polypropylene filters together by using the smoothed end of a stainless steel tube and pressing down on the filters and rotating slightly to form a circular ring of compression around the enclosed GF filter. Diffusion packets can be made several days ahead of use and stored in a bottle with a tight-fitting cap.
- Place a diffusion packet in each of the bottles.
- Add 0.35 g of MgO (precombusted to 475 °C) to each 60-mL bottle (1.0 g for 200 mL in a 250 mL bottle) and cap the bottle immediately.
- Secure the bottles in a rotating hybridization/incubation oven at 45 °C for 72 h (or longer as appropriate for larger sample volume; for example, 10 days for 200 mL sample in a 250 mL bottle).
- Remove the diffusion packets and place them in uncapped, individual, prebaked (475 °C for 4–5 h) glass scintillation vials. Place the vials in a vacuum oven for 2 h at 45 °C to remove outside moisture. Place the vials in a desiccator containing concentrated H_2SO_4 in a dish at the bottom of the vessel.
- After 24 h of drying in the desiccator, remove the vials, cap them, and analyze the contents within 2 weeks.

Appendix C. Excel Sample Workbook

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

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	N-6	N-6	N-6	N-8	N-8	N-8	N-6	N-8	N-13273	G-9033	G-9034	G-9035
	Set weight mg	0.13	0.13	0.13	0.13	0.13	0.13						
	Actual weight, mg	0.129	0.135	0.130	0.129	0.133	0.129						
	Comment	pure	pure	pure	pure	pure	pure	GF/C	GF/C	Diffusion blank	GF/C	GF/C	GF/C
B	Our Lab ID	G-9036	G-9037	G-9038	G-9039	N-6	N-8	G-9040	G-9041	G-9042	G-9043	G-9044	G-9045
	Set weight mg												
	Actual weight, mg												
	Comment	GF/C	GF/C	GF/C	GF/C	GF/C	GF/C	GF/C	GF/C	GF/C	GF/C	GF/C	GF/C
C	Our Lab ID	G-9046	G-9047	N-6	N-8	N-6	N-8						
	Set weight mg					0.13	0.13						
	Actual weight, mg					0.129	0.131						
	Comment	GF/C	GF/C	GF/C	GF/C	pure	pure						
D	Our Lab ID												
	Set weight mg												
	Actual weight, mg												
	Comment												
E	Our Lab ID												
	Set weight mg												
	Actual weight, mg												
	Comment												

Note. Pure = solid salt aliquot; GF/C = aliquot extracted from solution on glass fiber filter

Worksheet 2. Samples to be analyzed.

[International reference materials are in bold font]

Line	Identifier	Port	Comment	Amount	Amt Unit
1	N-6	A1	pure	0.129	mg
2	N-6	A2	pure	0.135	mg
3	N-6	A3	pure	0.130	mg
4	N-8	A4	pure	0.129	mg
5	N-8	A5	pure	0.133	mg
6	N-8	A6	pure	0.129	mg
7	N-6	A7	GF/C		mg
8	N-8	A8	GF/C		mg
9	N-13273	A9	diffusion blank		mg
10	G-9033	A10	GF/C		mg
11	G-9034	A11	GF/C		mg
12	G-9035	A12	GF/C		mg
13	G-9036	B1	GF/C		mg
14	G-9037	B2	GF/C		mg
15	G-9038	B3	GF/C		mg
16	G-9039	B4	GF/C		mg
17	N-6	B5	GF/C		mg
18	N-8	B6	GF/C		mg
19	G-9040	B7	GF/C		mg
20	G-9041	B8	GF/C		mg
21	G-9042	B9	GF/C		mg
22	G-9043	B10	GF/C		mg
23	G-9044	B11	GF/C		mg
24	G-9045	B12	GF/C		mg
25	G-9046	C1	GF/C		mg
26	G-9047	C2	GF/C		mg
27	N-6	C3	GF/C		mg
28	N-8	C4	GF/C		mg
29	N-6	C5	pure	0.129	mg
30	N-8	C6	pure	0.131	mg
31		C7			mg
32		C8			mg
33		C9			mg
34		C10			mg
35		C11			mg
36		C12			mg
37		D1			mg
38		D2			mg
39		D3			mg
40		D4			mg

Appendix D. Step-by-Step Procedure for Placing Sample Filters in Capsules and Weighing Reference Samples

1. Using gloves, open the 5×7 mm tin capsule to a flat circle.
2. Using forceps and a spatula, open the diffusion packet.
3. Place GF/C filter in the center of the tin circle and carefully fold the tin circle with filter in half.
4. Continue folding until the capsule is a compact sphere.
5. Place the sphere in a weighing tray in the same order as shown on the Excel worksheet (example on Worksheet 1).
6. Repeat steps 1 through 5 for every sample (in-house references as well as unknowns and a blank filter).
7. Insert Template diskette in the computer connected to the microbalance.
8. Condition the balance (this step should be done once a day):
 - a. Place the empty capsule on the balance and close the door. Wait for the reading to stabilize (the “mg” on the display appears). Tare the balance.
 - b. Remove and replace the capsule and make sure the stabilized weight is 0.000 mg.
 - c. Repeat these steps until the balance is stable.
9. Remove the capsule from the balance, add pure isotopic reference standards, and weigh the filled capsule. Repeat until the desired weight is achieved ($132 \pm 10 \mu\text{g}$).
10. Enter the sample weight on the template.
11. Fold the cup; secure the sample in it.
12. Repeat steps 4 to 7 for every pure reference sample.
13. Note:
 - a. Do not cross-contaminate samples.
 - b. Make sure the sample area and the spatula are cleaned using wipes between each sample.
14. Always allow the balance to stabilize before removing the capsule.

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

1. Before opening the lid, make sure the isolation valve (between the sample chamber and the 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 while holding the lid and open the lid.
4. Place the 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 the He purge isolation valve. 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 the sample chamber and He flow rate to stabilize. Close the He purge isolation valve.
8. Open the isolation valve (arrow toward up) and wait for 2 min for stabilized baseline. Normally when the emission current is set at 0.5 mA/0.48 mA with He carrier flow rate of 90 mL/min, you 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 K).

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

1. Insert the diskette with “Weighing Template” on it.
2. Open “Sequence File” under “EA Configuration.”
3. Open the “Weighing Template” in Excel.
4. “Copy” and “paste” the list of Sample ID, sample weights, and comments from template to sequence table.
5. Select appropriate “method” for each sample.
6. Define reference line and blank line.
7. Start.
8. Enter appropriate folder name and select pre: “Date.”
9. Give File Name: Select pre: “Analysis #.” Select post: “Identifier 1.”
10. Select: “Print Result.”
11. Click OK.
12. Wait for first sample to be analyzed.

Appendix G. Step-by-Step Procedure to Retrieve Data from ISODAT for LIMS-LSI and for Data Backup

1. For ISODAT:
 - a. Select “Results” in ISODAT.
 - b. Select the result folder you wish to export data from.
 - c. Select all the individual analyses you want to retrieve by right clicking the selected file.
 - d. Select “Reprocess.”
 - e. Give a file name.
 - f. Add the export template previously designed.
 - g. Open.
 - h. OK. (Reprocessing takes 1–2 min)
 - i. Open Excel.
 - j. Find the file you reprocessed.
 - k. Open it in Excel.
 - l. Define all reference peaks by adding “1” under column “Is Ref.?”
 - m. Transfer the data to a Zip disk or a “memory stick.”
 - n. Import the data to LIMS-LSI.
2. For Data Backup Computer:
 - a. Go to Windows Explorer in the ISODAT computer.
 - b. Find the drive where the data are.
 - c. Choose: “Finnigan.”
 - d. Choose: “User.”
 - e. Choose the inlet system where you have data (EA).
 - f. Choose “Results folder.”

Appendix H. Step-by-Step Procedure to Transfer Data to LIMS-LSI, Transfer Data to Data Backup Computer, and to Reevaluate Old Data

1. Transfer the 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 the data to the Data Backup computer:
 - a. Go to Windows Explorer in the Data Backup computer.
 - b. Find the drive your backups are stored.
 - c. Choose: "RSIL."
 - d. Choose: "Mass. Spec. Analysis Backup."
 - e. Choose: "P." (Stands for DeltaPlus 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 backups every 2 weeks or so, as required.
3. Reevaluate old data:
 - a. Find your samples in the Data Backup 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 I. 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 those references that were processed as aqueous diffusion samples.
7. Accept "Expansion Correction and Additive Correction factors."
8. Print out the correction factor sheet.
9. Report the daily reference values and the correction factors along with the date and the range of analysis number in the "EA" binder.
10. Go back to the LIMS-LSI main menu by closing any open windows.
11. Choose "Print Samples in Progress."
12. Open "Sample in Progress."
13. Choose an appropriate isotope.
14. Choose the appropriate prefix (for example, G for general, N for nitrogen).
15. Put in sample ID range of samples to be analyzed. Click "Print."
16. Review results; determine repeats.

Appendix J. 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 regulator counterclockwise.
4. If the gauge needle does not move, there is no leak.
5. If the gauge needle moves, indicating a loss 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 K. Daily Checklist

Analyst in charge: _____ Date: _____

1. Weekly:

- Change the working reference gas, O₂ 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.
- Check and clean the autosampler carousel.
- Change the reaction tubes (oxidation, reduction) after approximately 250 samples or when necessary.
- Change the water trap after approximately 200 samples or when necessary.

2. Daily

- Check He, O₂, and reference gas flow. []
- Check EA reaction and reduction tubes temperature (1020 °C and 650 °C). []
- Change the insertion tube (ash collector liner) in the reaction tube. []
- Check background masses. []

Typical Background at N₂ 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	1 × 10 ¹¹	2

- Perform “Ref on/off” method three times to stabilize IRMS. []
- Check the peak center. If it is off default value by 50, do mass calibration. []
- Analyze “Ref on/off” method 10 times to stabilize IRMS (std should be < 0.1 ‰). []

ConFlo Pressure Setting N ₂	N ₂ signal (V) Mass 29 on Cup 2
0.25	0.173
0.50	0.450
0.75	0.773
1.00	1.180
1.25	1.560
1.50 Optimal setting N₂	2.074
1.75	2.634
2.00	3.199

- Check the paper in printer. []

Appendix L. Changing the Insertion Tube

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

1. Manually close the needle valve near the ion source.
2. On the menu of the Model 2500 EC under “Sp. Fun.,” choose “Std By,” and press “Enter.” The O₂ 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 a clean tube from the drawer that has 0.5 cm of quartz wool packed on the bottom and lower it down with the wire tool.
6. Replace the carousel and tighten the valves with a wrench.
7. Turn the O₂ and He back on by pressing “Enter” and return the screen to the temperature readout by pressing “Sp. Fun.”
8. Open the needle valve.

Appendix M. 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 V 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 approximately 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 the used water trap 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 decrease in pressure to check for a leak in the system. If everything looks acceptable after 5 min, replace the vent line.
7. Under the “Sp. 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 this tube to the water trap bag in the drawer.

Appendix N. Changing the Reaction Tube

The reaction tube should be changed approximately every 200 to 250 samples.

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 reaction tube temperature to less than 900 °C.
4. Retrieve the reaction tube from the cabinet.
5. Insert the insertion tube into the top of the reaction tube; there will be a plastic tube in the reaction tube that should be removed. The bottom of the reaction tube has copper in it.
6. Place a black O-ring on the top of the reaction tube. Make sure that all O-ring connections are clean and free of any dust.
7. Loosen the top metal seal and remove the bottom metal seal. Go back and completely remove the top seal. It will be very hot! Use oven gloves!
8. Remove the quartz reaction tube and place it in a metal can, place the clean reaction tube in the column. Make sure the O-ring is clean; then replace the top.
9. On the bottom, put on the metal piece with the metal O-ring first and then add the clean rubber O-ring. Raise the bottom portion and screw together. Then, tighten the connection with wrenches and put the metal support back in place.
10. Turn the gases back on; then go to “CNT” in the menu under “Sp. Fun.” Set to zero the counter of the reaction tube “B.”
11. Insert the plug into the vent and close the He supply. Watch for any decrease in the pressure to check for a leak in the system. If everything looks okay after 3 min, open the He supply and replace the vent line.
12. Open the needle valve.

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

1. Open “Store Samples in Progress” in LIMS-LSI.
2. Choose the appropriate isotope.
3. Choose sample ID range from “Sample in Progress” print out.
4. Store data.
5. Go back to the 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 the 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 related to this project.
10. If the samples were submitted through the NWQL, click “NWQL Export” and transfer the data in NWQL format to a diskette; report these data to the NWQL by e-mail. Print a copy of the NWQL export file and save it in the “Correspondence” binder with the project report printed in step 9.