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Determination of the $\delta^{13}C$ of Dissolved Inorganic Carbon in Water; RSIL Lab Code 1710

Chapter 18 of
Section C, Stable Isotope-Ratio Methods
Book 10, Methods of the Reston Stable Isotope Laboratory

Techniques and Methods 10–C18

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By Glenda L. Singleton, Kinga Révész, and Tyler B. Coplen

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KEN SALAZAR, Secretary

U.S. Geological Survey
Marcia K. McNutt, Director

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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 Fractionation in Hydrologic Processes 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 hydrogen, nitrogen, oxygen, carbon, and sulfur 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 water-supply sustainability, groundwater/surface-water interactions, paleoclimate history, biologic cycling of nutrients, groundwater 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 Glynn
Chief, Branch of Regional Research, Eastern Region

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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 (kg) = 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 (°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
IAEA	International Atomic Energy Agency
DIW	deionized water
DI-IRMS	dual inlet isotope-ratio mass spectrometer
DIC	dissolved inorganic carbon

LIMS-LSI	Laboratory Information Management System for Light Stable Isotopes
LN_2	liquid nitrogen
L-SVEC	lithium SVEC (name of lithium carbonate reference material)
m/*z*	mass-to-charge ratio
NBS	National Bureau of Standards
NFPA	National Fire Protection Association
per mil, ‰	part per thousand (1/1000)
pF	picofarad
PTFE	polytetrafluoroethylene
QA	quality assurance
QC	quality control
QC/QA	quality control/quality assurance
RM	reference material
RSIL	Reston Stable Isotope Laboratory
SOP	standard operating procedure
std	international measurement standard
USDA	U.S. Department of Agriculture
USGS	U.S. Geological Survey
VPDB	Vienna Peedee Belemnite

Symbols

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

Determination of the $\delta^{13}C$ of Dissolved Inorganic Carbon in Water; RSIL Lab Code 1710

By Glenda L. Singleton, Kinga Révész, and Tyler B. Coplen

Summary of Procedure

The purpose of the Reston Stable Isotope Laboratory (RSIL) lab code 1710 is to present a method to determine the $\delta^{13}C$ of dissolved inorganic carbon (DIC) of water. The DIC of water is precipitated using ammoniacal strontium chloride ($SrCl_2$) solution to form strontium carbonate ($SrCO_3$). The $\delta^{13}C$ is analyzed by reacting $SrCO_3$ with 100-percent phosphoric acid (H_3PO_4) to liberate carbon quantitatively as carbon dioxide (CO_2), which is collected, purified by vacuum sublimation, and analyzed by dual inlet isotope-ratio mass spectrometry (DI-IRMS). The DI-IRMS is a DuPont double-focusing mass spectrometer. One ion beam passes through a slit in a forward collector and is collected in the rear collector. The other measurable ion beams are collected in the front collector. By changing the ion-accelerating voltage under computer control, the instrument is capable of measuring mass/charge (*m/z*) 45 or 46 in the rear collector and *m/z* 44 and 46 or 44 and 45, respectively, in the front collector. The ion beams from these *m/z* values are as follows: *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. The data acquisition and control software calculates $\delta^{13}C$ values.

Reporting Units and Operational Range

Variations in isotopic compositions typically are small. Stable isotope ratios commonly are measured and expressed as the relative difference ratio of the number of the less abundant isotope (usually the heavy isotope) to the number of the more abundant isotope (usually the light isotope) of a sample with respect to the measurement standard. This relative difference is designated $\delta^i E$, which is the shortened form of $\delta(^iE/^jE)$ or $\delta(^iE)$, and is defined according to equation 1 (Coplen, 2011):

$$\delta^i E = \delta\left(^i E\right) = \delta\left(^i E/{}^j E\right) = \frac{N\left(^i E\right)_P / N\left(^j E\right)_P - N\left(^i E\right)_{std} / N\left(^j E\right)_{std}}{N\left(^i E\right)_{std} / N\left(^j E\right)_{std}} \quad \textbf{(1)}$$

where $N(^iE)_p$ and $N(^jE)_p$ are the numbers of the two isotopes ^iE and ^jE of element E in specimen P, and equivalent parameters follow for the international measurement standard, "std." A positive $\delta^i E$ value indicates that the unknown is more enriched in the amount of the heavy isotope, ^iE, than the std is. A negative $\delta^i E$ value indicates that the unknown is depleted in the amount of the heavy isotope, ^iE, relative to that of the std. For stable carbon isotopes, $\delta^{13}C$ is defined as follows:

$$\delta^{13}C = \delta\left(^{13}C\right) = \delta\left(^{13}C/{}^{12}C\right) = \frac{N\left(^{13}C\right)_P / N\left(^{12}C\right)_P - N\left(^{13}C\right)_{std} / N\left(^{12}C\right)_{std}}{N\left(^{13}C\right)_{std} / N\left(^{12}C\right)_{std}} \quad \textbf{(2)}$$

The reference materials for relative carbon isotope-ratio measurements ($\delta^{13}C$) are NBS 19 $CaCO_3$ and L-SVEC Li_2CO_3. By interspersing internationally distributed isotopic reference materials with accepted δ values among samples with unknown δ values, the δ values can be determined on normalized $\delta^{13}C$ scales. The system was tested by analyzing carbonate with $\delta^{13}C$ values ranging between approximately –50 and +3 ‰.

Routine analysis requires a 35-mg sample of $SrCO_3$ to react with 2 mL of H_3PO_4 (McCrea, 1950). The CO_2 produced by this reaction is purified to remove water, transferred to a glass sample tube, and analyzed for $\delta^{13}C$ content. The precision and accuracy of the results are typically ±0.1 ‰ for $\delta^{13}C$ measurements. All reported results are sent to the submitter as an Excel file with an accompanying report briefly explaining the method. In some cases, a minimum mass of sample of 3 mg may suffice.

Reference Materials and Documentation

Reference Materials Used, Storage Requirements, and Shelf Life

In this method, the international measurement standards for $\delta^{13}C$ measurements are NBS 19 $CaCO_3$ and L-SVEC Li_2CO_3, which have consensus $\delta^{13}C$ values of +1.95 ‰ and –46.6 ‰, respectively, on the Vienna Peedee Belemnite (VPDB) scale (Hut, 1987; Coplen, 1996; Coplen and others, 2006). A locally calibrated carbonate (VP, Vicki Prindle) was prepared by thoroughly crushing marble to pass through a 100-mesh (149-µm) sieve and collected on a 200-mesh (74-µm) sieve; it has a $\delta^{13}C$ value of +1.35 ‰. All particles <200 mesh were discarded. Fine dust was removed by washing with acetone and drying in an oven at 90 °C overnight. All of these standards are stored at the RSIL in glass bottles with Polyseal conical insert caps to keep moisture out. Their shelf life is indefinite. These standard materials have a wide range of $\delta^{13}C$ values and are analyzed routinely with unknown samples. Carbon isotopic compositions of carbon-bearing, internationally distributed isotopic reference materials used in this technique are shown in the Documentation section (table 1).

Documentation

All calibration results are stored in the Laboratory Information Management System for Light Stable Isotopes (LIMS-LSI; Coplen, 2000) using sample identifiers with the prefix of C (see table 1, column 4.)

Table 1. Carbon isotopic reference materials.
[RSIL, Reston Stable Isotope Laboratory; VPDB, Vienna Peedee Belemnite]

Description	Compound Name	$\delta^{13}C_{VPDB}$	RSIL ID
NBS 19	Calcium carbonate	+1.95 ‰	C-4800
NBS 18	Calcium carbonate	–5.01 ‰*	C-2493
IAEA-CO-1	Calcium carbonate	+2.49 ‰*	C-10828
IAEA-CO-8	Calcium carbonate	–5.76 ‰*	C-10829
L-SVEC	Lithium carbonate	–46.6 ‰	C-4182
IAEA-CO-9	Barium carbonate	–47.32 ‰*	C-9477
In house (VP)	Calcium carbonate	+1.35 ‰*	C-9634

*Laboratory-assigned value based on Commission on Isotopic Abundances and Atomic Weights (CIAAW, 2010).

Labware, Instrumentation, and Reagents

Preparatory labware and materials include glass carbonate reaction vessels and stopcock-equipped tops, glass sample tubes with fitted stopcock attachments, a 2,500-µL pipetter with pipette tips, vessel holding rack, 47-mm Millipore filtering apparatus, Millex-HA 0.45-µm filter units, 1-L vacuum flasks, a tank of compressed nitrogen gas with regulator, a peristaltic pump, glass Petri dishes, a 60-mesh (250-µm) sieve, rubber bands, tweezers, tissues, weighing paper, and an analytical balance capable of measuring samples to 0.1-mg accuracy.

The preparatory line consists of three high-vacuum lines, which are all connected to a rotary rough pump and a mercury diffusion pump capable of achieving a vacuum of 5 mTorr. The three parts that comprise the preparatory line are (1) a sample tube cleaning line, (2) a 10-sample-port manifold, and (3) a pentamanifold. These parts are used as follows:

1. The sample tube cleaning line has heaters that can be lowered over eight sample tubes simultaneously. Dirty sample tubes are heated for 1 hour under vacuum to remove moisture, other contaminants, and CO_2 adsorbed on the glass walls of the sample vessel before use.
2. Before reacting the carbonate with acid, the 10-sample-port manifold should be used to evacuate air at ambient temperature from the glass sample reaction vessels containing carbonate samples and H_3PO_4.
3. The third preparatory vacuum line is a three-tiered, cylindrical, five-sample-port manifold (pentamanifold) with two sets of cold fingers facing the center of the manifold on the second and third tiers. These small fingers are used for isolating and cryogenically purifying CO_2 from the reaction vessels and transferring the pure CO_2 to sample tubes for analysis. The pressure of each unknown gas sample is measured by cryogenically trapping samples in the center-facing fingers of the third-tier pentamanifold, which are connected to pressure transducers. A meter reading between 15 and 300 translates to an adequate amount of gas for analysis with the mass spectrometer indicated below. After gas samples are cryogenically trapped in sample tubes on the bottom tier, stopcocks of the glass sample vessels are closed, and the samples are transferred to the gas handling system for analysis by DI-IRMS.

The isotopic analytical apparatus consists of three major components: (1) gas handling system connected to the DI-IRMS, (2) DI-IRMS, and (3) computer software.

1. The gas handling system is constructed with two stainless steel manifolds (fig. 1), each connected to 10 sample-port valves, accommodating 19 sample tubes. The samples are connected to the DI-IRMS sample piston and pneumatically actuated valves are opened one at a time to release each sample to the sample piston. Each pneumatically actuated value is controlled by solenoid valves controlling compressed air (5×10^{-6} kPa).
2. The DI-IRMS is a modified DuPont 491 mass spectrometer (fig. 2) with a double-collecting Faraday cup collector (Coplen, 1973). In the DI-IRMS, gas molecules are ionized in a source by electrons emitted from a hot filament. The ions are accelerated into an energy analyzer, separated in a magnetic field, and collected in Faraday cup collectors. This ion geometry provides double focusing of ions. The ion-beam intensities are measured with electrometers. The feedback resistors on the major and minor cup electrometers are approximately 1×10^{9} Ω and 5×10^{10} Ω, respectively. The response speeds of each electrometer are matched by slowing the response speed of the major electrometer with a resistor-capacitor response-speed filter. The mass spectrometer has an ion-beam monitor located between the energy analyzer and the magnetic analyzer, which provides the intensity of the total ion current. The ratio of the beam monitor current to the $m/z = 44$ current for samples and reference gases gives a good determination of

sample contamination. Each sample is loaded into a computer-controlled sample piston (Coplen, 1981). One computer-controlled, working-reference gas piston feeds gas into the reference capillary.

3. The Microsoft Visual Basic data acquisition and control software is designed to (1) control the gas handling unit, (2) control the DI-IRMS, (3) acquire data from the DI-IRMS, and (4) process and store data directly to the LIMS-LSI.

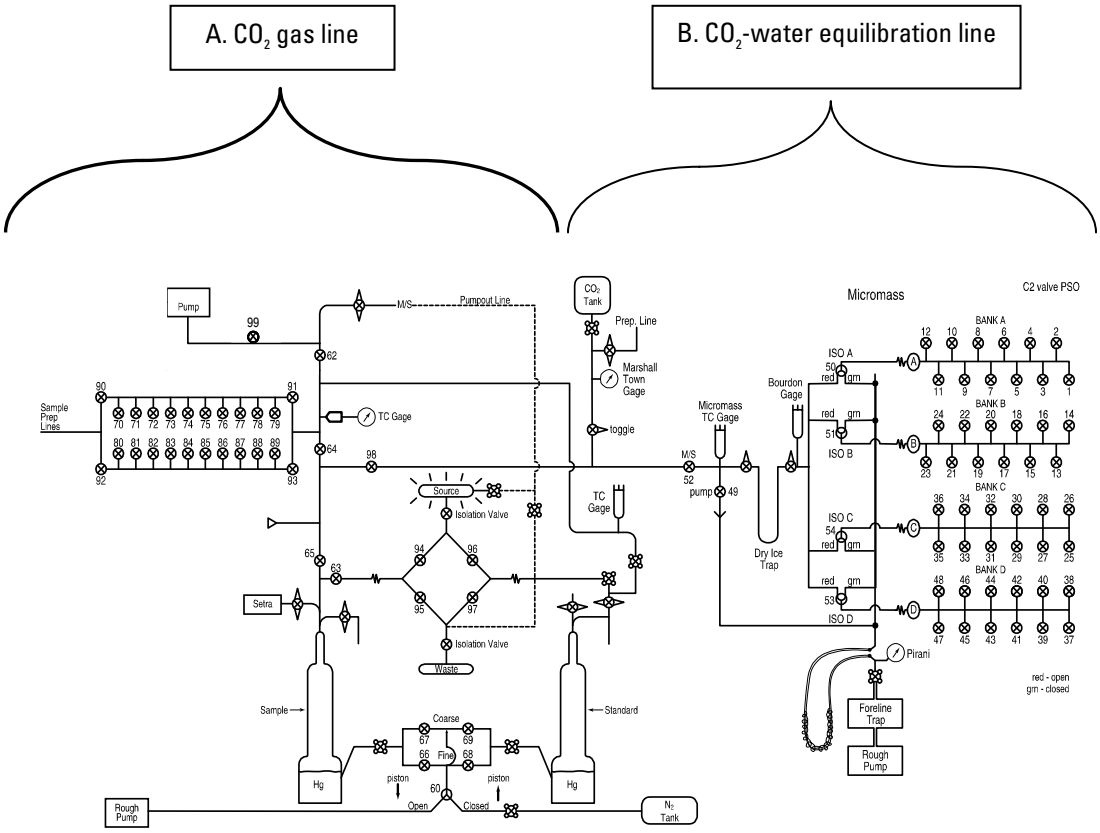

Figure 1. Schematic of the A, CO_2 gas line and B, CO_2- water equilibration line (not used for determination of the $\delta^{13}C$ of DIC in water).

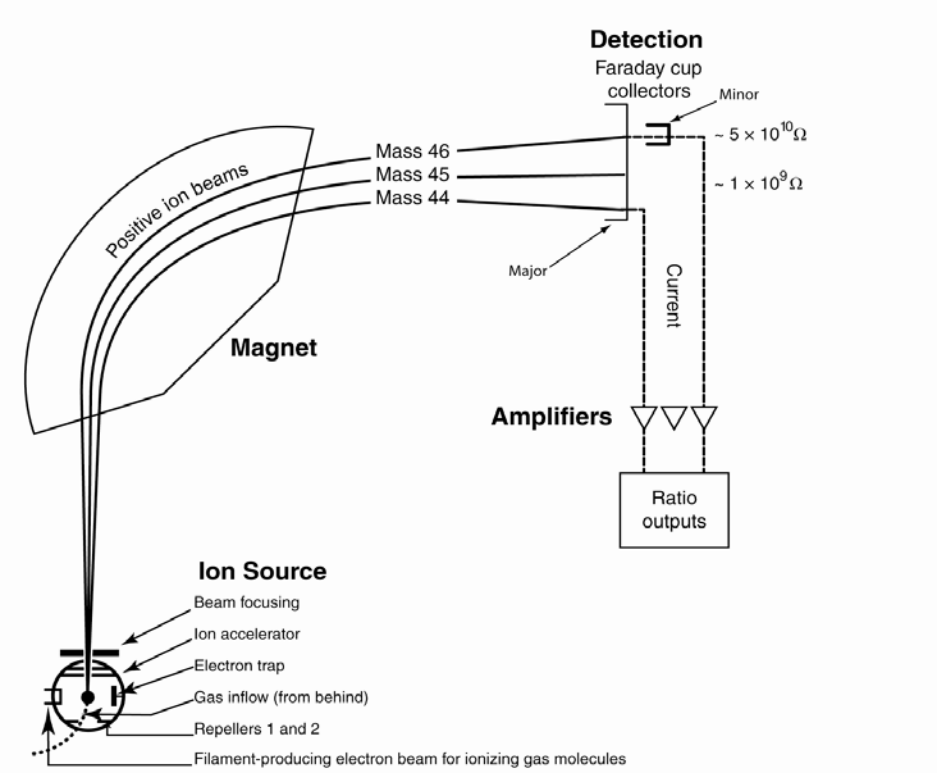

Figure 2. Schematic of a dual-inlet isotope-ratio mass spectrometer (DI-IRMS) (modified from Clark and Fritz, 1997).

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

Sample Containers, Preservation, and Handling Requirements

Sample preparation involves using the LIMS-LSI system for logging in sample batches that contain 1 to 100 samples per batch (also called a project). A step-by-step procedure to log in samples to LIMS-LSI is given in appendix A. Projects need labels printed for each sample and need a printed 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." The time requirement for this procedure is approximately 15 minutes to 2 hours, depending on sample quantity and level of organization.

Sample Preparation and Time Requirements

Each sample is collected in a glass bottle with a volume between 250 mL and 1 L, depending on the expected DIC concentration. If DIC concentrations are unknown, at least 1 L is collected and processed. The bottles are paired with a Polyseal conical insert cap to minimize atmospheric CO_2 contamination. If Polyseal caps are not available, bottle lids should be sealed with Parafilm or electrical tape to help make the container air tight.

The minimum amount of sample required is that which is used to yield approximately 50 mg of $SrCO_3$ (dry weight). However, a larger mass of sample is desirable. Bottles are labeled with isotopes to be analyzed, and they are labeled with a unique sample ID. In the field, sample bottles should be filled with

sample by overflowing directly from the sample collection device into the bottle. Approximately 15 mL of sample is poured from the full bottle, and DIC of the water sample is precipitated by the addition of ammoniacal $SrCl_2$ (1 mL per 100 mL of sample). The bottle is immediately capped. The protocol for the preparation of ammoniacal $SrCl_2$ can be found in appendix B. The DIC in the sample reacts with the ammoniacal $SrCl_2$ to form $SrCO_3$, an easily extractable precipitant. The sample is allowed to precipitate at ambient temperature for at least 24 hours before further sample processing takes place. If ammoniacal $SrCl_2$ is not added to the sample at the time of collection, the sample should be refrigerated at 4 °C until ammoniacal $SrCl_2$ can be added. This step minimizes microbial activity, which has the potential to alter the isotopic composition of the sample. After precipitation of $SrCO_3$, the sample can be stored at ambient temperature.

A step-by-step procedure of the filtering of samples is given in appendix C. After samples have been treated with ammoniacal $SrCl_2$, they are filtered through 0.45-µm glass filters to separate the $SrCO_3$ from the water and any excess ammoniacal $SrCl_2$ in the sample bottle. A peristaltic pump is attached to a filter flask just below the filter tube, pulling the sample across the filter. The filter tube cap fitting above the sample provides a flow of positive-pressure nitrogen gas, helping to minimize air exposure and helping to force the water through the filtering system. This closed system arrangement ensures quick collection of the sample on the filter paper and quick elution of the liquid sample while limiting exposure to extraneous CO_2.

The sample with precipitate is poured incrementally (approximately 10–20 mL at a time) through a filter tube of either a short or long length. The shorter tube length is used for samples with a relatively high quantity or very fine $SrCO_3$ precipitate. The longer tube length is used for samples with less $SrCO_3$ precipitate. The sample is collected on the filter paper and then flushed with at least 5 L deionized water (DIW) to bring the pH of the carbonate to a neutral level (pH between 7 and 8). After 5 L of water have passed through the $SrCO_3$ on the filter, the pH is checked by testing the flow-through water from the filtering tube. If the pH of the flow-through water is neutral, then the filtering system is disassembled, and the precipitate on the filter paper is checked for pH neutrality using litmus paper. The sample is continuously flushed with DIW until the precipitate has a neutral pH value. Once the pH is neutral, the filter paper with sample is transferred to a glass Petri dish and dried overnight at 90 °C. The dried sample is then isotopically homogenized by sieving through a 250-µm sieve. This step is crucial because a substantial kinetic isotopic fractionation occurs when $SrCO_3$ is precipitated. A representative sample for analysis needs to come from a well-homogenized sample source. The homogenized sample is placed in a dry, clean, air-tight vial, which is labeled with the corresponding RSIL Lab ID. The $SrCO_3$ sample is now ready for reacting with H_3PO_4. A log of the following procedure of the samples is recorded using a "Carbonate Sample Record Sheet." An example can be found in appendix D.

Samples are weighed out (approximately 35 mg) and placed into properly labeled glass carbonate reaction vessels. The carbonate is placed into the main body of the vessel, and 2 mL of 100-percent H_3PO_4 (see appendix E for the procedure for H_3PO_4 preparation) is added to the side arm of each glass reaction vessel. The vessels are then put on the high-vacuum line and the air is evacuated from the chamber. Once evacuated, the stopcock of the reaction vessel is closed, and the reaction vessels are removed from the high-vacuum line. The samples are reacted with the H_3PO_4 by tipping the vessels and allowing the acid to flow into the main body of the vessel. Each vessel is then placed upright, and samples are reacted at least overnight.

After reaction with the acid, CO_2 is extracted from the reaction vessels, cryogenically purified, and transferred to glass sample tubes using the three-tiered pentamanifold vacuum line. The CO_2 sample tubes are then transferred from the pentamanifold to the gas handling system where they should be ready for analysis using DI-IRMS. The step-by-step procedure of CO_2 purification is printed and kept in the "Lab Procedures" binder. The supervisor reviews any suggested changes before they are instituted. The current procedure is given in appendix F.

Performing the Analysis and Time Requirements

Before starting isotopic analyses of the samples, the analyst ensures the mass spectrometer and preparation system are functioning properly by checking off items in pen on the "CO_2 Mass Spectrometer Checklist" (appendix G). This document is replaced weekly and saved for analytical audits. A new column of the checklist is filled out each day the apparatus runs. Another form is used to keep a record of the samples analyzed daily. This form, "DuPont Sample Record Sheet" (appendix H), is placed on a clipboard underneath the gas handling system. Any special comments are recorded on this daily record, which is saved for laboratory audits.

The isotopic measurement is computerized. As each sample is about to be analyzed, pneumatically actuated valves are opened to expand the sample gas into the sample piston for 12 seconds. Approximately 70 percent of the CO_2 from a sample vessel is expanded into the glass sample piston. Pressure adjustment of the sample piston is performed with an absolute pressure transducer; finer adjustment is performed with the $m/z = 44$ ion-beam voltage. Samples are analyzed sequentially. The $\delta^{13}C$ values are normalized to the VPDB–L-SVEC scale by the mass spectrometric software. The calculated delta value is called the penultimate delta and it is saved in real time in the LIMS-LSI Microsoft Access database.

An analyst at the LIMS-LSI determines daily correction factors using the $\delta^{13}C$ values of the reference materials using equations similar to the following:

$$-46.6\ ‰ = m \times (\delta^{13}C_{C\text{-}4182,VPDB}) + b \quad \textbf{(3)}$$

$$1.95\ ‰ = m \times (\delta^{13}C_{C\text{-}4800,VPDB}) + b \quad \textbf{(4)}$$

The values, –46.6 ‰ and 1.95 ‰, are the consensus values of the international measurement standards L-SVEC Li_2CO_3 and NBS 19 $CaCO_3$, respectively. Samples are normalized to the VPDB–L-SVEC scale by assigning values of these international standards. The $\delta^{13}C$ values in equations 3 and 4 are the mean delta values of the respective reference in a sample analysis sequence relative to VPDB; b is the additive correction factor, and m is the expansion coefficient correction factor. The procedure for applying correction factors is given in appendix I.

All of the samples are prepared and analyzed in duplicate. If the replicates do not agree within acceptable tolerances, they are prepared and analyzed until acceptable statistics are achieved. Without special attention, the computerized LIMS-LSI will not allow carbon isotopic results to be stored if they are outside of two-sigma uncertainty. All reported results are within this range, unless otherwise indicated. The time requirement for performing the analyses described above is a minimum of 4 days.

Problematic Samples

Problematic samples include those that contain sulfate. The SO_2 produced during the acid reaction readily freezes with CO_2 at liquid nitrogen (LN_2) temperature, causing contamination in the sample during the cryogenic purification step.

Small samples are problematic when insufficient CO_2 gas is produced for analysis by DI-IRMS. With small samples, the entire sample, including the small amount of precipitate that may remain on the filter paper, is needed for analysis, and there is no need for the homogenization step in which the sample is passed through the sieve. In this case, the entire filter paper with all precipitate is reacted with acid directly. The filter paper is made of glass microfiber and will not react with H_3PO_4.

Interferences

If stopcock grease from glass sample vessels is cleaned with acetone and this acetone contaminates a CO_2 sample, it appears as a contribution to the *m/z* 45 beam. Therefore, acetone should not be used to remove stopcock grease. The use of trichloroethylene is recommended to remove stopcock grease from laboratory glassware.

Troubleshooting and Bench Notes

If a satisfactory vacuum cannot be attained during sample preparation on any of the vacuum lines, the analyst should leak check the system and correct the problem. After every set of analyses, the analyst should inspect data sheets. If a port is contaminated or the sample is too small, the analyst should make a note of the port number and notify the supervisor.

Maintenance and Maintenance Records

Before use, glass sample tubes are baked under vacuum at 230 °C for 1 hour on a vacuum line, which is specially built to evacuate and heat eight sample tubes simultaneously. This procedure removes CO_2 from a previous sample that might be adsorbed on the inner glass surface of the tube. Leaking sample tubes are marked and their stopcocks removed, cleaned with trichloroethylene, regreased, and leak tested before use. From time to time, the ports of the sample manifold connected to the DI-IRMS are checked by adding isotopically homogeneous CO_2 gas uniformly to all the ports, and these gas samples are analyzed for their $\delta^{13}C$ value. The standard deviation of the measurement results should be $<\pm 0.1$ ‰.

Maintaining the vacuum lines is essential. This includes occasionally checking pump performance, changing oil in rotary pumps every 6 months, adding mercury to the diffusion pump, removing mercury from the diffusion pump cold traps, and cleaning and greasing stopcocks on a regular basis. Pump conditions, such as date of oil change, problems, and repairs, are listed in a pump logbook, as well as in a database on the laboratory main computer (File path: LIMS C:\RSIL\vacuum pumps). The mass spectrometer does not require regular maintenance; however, it does require a daily maintenance check, which is performed by the analyst when starting a new run of samples on the mass spectrometer (see appendix G). A logbook is kept for each mass spectrometer, where notes about maintenance checks, normal settings, problems, and repairs are listed.

Sample Retention Time and Disposal

Samples are retained in the RSIL for at least 6 months after reporting results to sample submitters. Samples are then discarded unless the submitter has requested that samples be returned.

The sample-analysis file from the DI-IRMS computer is kept indefinitely on two different hard disks of the data back-up computer. Paper reports of the results of isotope-ratio mass spectrometric analyses of the samples are kept indefinitely. Analytical results from the DI-IRMS (DuPont) are transmitted 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 Isotope (LIMS-LSI)

In the RSIL, we use the LIMS-LSI, created by T.B. Coplen (2000), 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 isotope-ratio analyses of samples, (3) calculating analytical results using standardized algorithms stored in a database, (4) normalizing stable isotopic data using isotopic reference materials, and (5) generating templates for convenient sample placement to facilitate loading of automated mass-spectrometer sample preparation manifolds. With this system, the following are ensured: (1) quality assurance, (2) laboratory efficiency, (3) reduction of workload and manual data-entry-related errors, 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 as an audit trail for the RSIL.

Quality Control (QC) Samples

Samples are analyzed in groups, with a maximum of 19 samples per day. Each group includes at least one pair of laboratory references that have been prepared and reacted at the same time as the unknown samples. One of these references is depleted in ^{13}C and the other has a moderate ^{13}C concentration with the intention of flanking the $\delta^{13}C$ values of the unknown samples.

Daily, an analyst examines the mass spectrometric analysis reports for problems and applies daily additive correction and expansion factors, which are determined with LIMS-LSI and applied to the isotopic data. After printing the 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 statistics (for example, $\Delta\delta$ should be less than or equal to 0.2 ‰).

Every few years, the RSIL participates in an International Atomic Energy Agency (IAEA) interlaboratory calibration. This serves as a calibration check of the mass spectrometer, the laboratory reference materials, and the computer data-management systems.

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 <0.15 ‰, use the mean δ.
- If there are three or more analyses, delete the outlier and recalculate the mean.
- If the standard deviation of this recalculation is <0.15 ‰, use the mean from this recalculation.
- Otherwise, set the value to null and reanalyze the sample.

The expanded uncertainty ($U = 2\mu_c$) obtained from long-term $\delta^{13}C$ measurement results is ±0.2 ‰, unless otherwise indicated. Expanded uncertainty in isotopic measurements is discussed by Coplen and others (2006). This means that if the same sample were resubmitted for isotopic analysis, the newly measured value would lay within the uncertainty bounds 95 percent of the time.

Corrective Action Requirements

If the 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 reproducible results after three or more separate analyses, the analyst averages all the data and reports the mean value. This value is indicated with a comment, and the customer will be advised by e-mail or other means.

Responsible Parties for All Quality Assurance/Quality Control Functions for Procedures Covered in RSIL Standard Operating Procedures

The analyst, with supervisory 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 on a project-by-project basis, reports results to the customers, and files final project data reports in the laboratory "Correspondence" binder. Step-by-step instructions are listed in appendix J. Customers are given their results in Excel format and are provided with an explanation of the analysis procedure (appendix K).

Health, Safety, and Waste-Disposal Information

Applicable Health and Safety Issues

Personal Protection

Safety glasses and protective latex gloves must be worn whenever vacuum lines or hazardous elements and chemicals are used in the isotope laboratory. H_3PO_4 and phosphorus pentoxide (P_2O_5) should be handled with nitrile gloves. When the submitter indicates hazardous chemical in the samples, consult the Material Safety Data Sheets (MSDS), which are on file in the laboratory and at the URL *http://www.ilpi.com/msds/# Manufacturers.* This URL provides links to the MSDSs of most chemical companies. Depending on the hazardous chemical, nitrile gloves may be needed.

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). For more information, consult the U.S. Geological Survey's *Safety and Environmental Health Handbook* (U.S. Geological Survey, 2002, 4–4.1).

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

Liquid nitrogen is used for separation of CO_2 from H_2O and in diffusion pump cold trap dewars. Mercury is used in diffusion pumps and in the pistons of the DI-IRMS. H_3PO_4 and P_2O_5 are also potentially dangerous and are used during the CO_2 production step and during the preparation of H_3PO_4. All samples should be carefully inspected on receipt for obvious indications of hazards. When the submitter indicates hazardous chemicals in the samples, consult the MSDS about handling the sample.

Gas Cylinder Handling

Compressed gas cylinders must be handled and stored according to U.S. Geological Survey's *Safety and Environmental Health Handbook* (U.S. Geological Survey, 2002, 4–4.5.1. 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

The U.S. Department of Agriculture (USDA) requires that unused soil or plant samples from areas outside the continental United States be autoclaved or otherwise treated to kill biologically active organisms before disposal. Follow the rules in USDA Circular Q–330.300–1 at *http://www.geotech.org/survey/geotech/pdf/soil-circular.pdf.* At the RSIL, samples are autoclaved with steam for 30 minutes at a pressure of 15 lb/in^2.

Trichloroethylene used for degreasing glassware is discarded in a properly labeled hazardous-waste collection bottle and recovered by the safety, health, and environment officer for disposal. Concentrated acid should be neutralized before disposal.

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

For samples submitted directly to the RSIL:

1. For sample submitter:
   a. Download "Standard Submission Excel Form" from the RSIL Web site at *http://isotopes.usgs.gov/*.
   b. Fill out the requested sample information.
   c. Send a diskette or CD and a hard copy along with the samples in addition to emailing RSIL personnel a copy of the submission file.
2. For RSIL personnel:
   a. Match up information on the sample bottles with the submitted Excel Worksheet.
   b. Enter all Excel Worksheet information into LIMS-LSI by loading the diskette. The submission date is the date samples are logged in.
   c. Use "Import New Project" in LIMS-LSI to assign C-#s; field IDs are the Station IDs.
   d. Print out one project report and label it for individual bottles.
   e. Put a label on a sample bottle and crosscheck Field IDs between bottles and Excel Worksheet forms.
   f. Punch holes in the original Excel Worksheet and all the information for the project and put it in the Samples-in-Progress binder.
   g. Put samples in the cabinet for storage until analyzed.

Appendix B. Procedure for Ammoniacal Strontium Chloride ($SrCl_2$) Preparation

Ammoniacal $SrCl_2$ is used to precipitate DIC in the sample to a $SrCO_3$ precipitate, which can be filtered from the liquid. Ammoniacal $SrCl_2$ is prepared by dissolving $SrCl_2$ in ammonium hydroxide (NH_4OH). The total process should take 10 minutes of preparation, plus 2 days of settling time.

Materials

- $SrCl_2 \cdot 6_2O$ 170 g
- NH_4OH 750 mL

Notes

Ammoniacal $SrCl_2$ can be prepared in the quantity shown under Materials or in any 4:1 molar ratio of NH_4OH to $SrCl_2$.

Procedure

1. In a glass reagent bottle, stir $SrCl_2$ into the concentrated NH_4OH solution using a swirling action until it is dissolved.
2. Replace the cap on the bottle tightly.
3. Let the bottle stand for 2 days.
4. Decant the clear reagent into a clean glass field reagent bottle. The precipitate in the bottom of the reagent is caused by exposure of CO_2 to the air; therefore, always use the clear reagent in the upper part of the field reagent bottle.
5. Seal the bottle cap with black electrical tape to prevent air CO_2 contamination and to prevent the cap from loosening.

Appendix C. Procedure for Filtering Strontium Carbonate ($SrCO_3$)

$SrCO_3$ is the material of interest and is to be filtered from the water sample. It should appear as a white precipitate at the bottom of the sample container.

Materials

- Sample to be prepared
- Filtering device; unit includes filter tube with O-rings, cap and bottom attachment
- 47-mm Millipore-gridded, stainless steel filtering membrane
- Type HA 0.45-µm Millipore filter papers
- 2-L vacuum flask
- 1-L flask
- Tank of compressed nitrogen gas with a pressure regulator
- Watch glasses or Petri dishes
- Sieve with mesh openings of 250 µm
- Glass vials for sample storage
- Litmus paper
- Peristaltic pump with tubing to fit the vacuum flask
- Oven, set at a temperature of 90 °C

Notes

The following should be set up under the fume hood where the filtering procedure is to be performed.

Vacuum System

One vacuum flask ≥2 L volume is fitted with a blue Millipore stopper with a gridded Millipore membrane. This will later accommodate the filter paper and filtering device. The filtrate from the filtering unit will collect in this flask. This flask is rinsed with 10-percent hydrochloric acid and DIW after filtering each sample to minimize contamination of subsequent samples in the event that the filter paper breaks and the filtrate needs to be refiltered using the contents of the flask.

A peristaltic pump with appropriately sized tubing is attached to the arm of the flask. Turn on the peristaltic pump and check the vacuum by placing a finger over the hole in the blue Millipore stopper on the flask. After a positive check for the vacuum, turn off the peristaltic pump.

Nitrogen Pressure Setup

Positive pressure from compressed nitrogen gas feeds through the top of the filtering device and is used to accelerate filtering and to minimize CO_2 contamination from atmospheric CO_2. Place the tank of compressed nitrogen gas near the fume hood. Be sure the tank is secure and handle the tank with care. Install a gas regulator with a long hose attached to the regulator nozzle. Attach the other end of the hose to the top lid of the filtering device and secure with a hose clamp.

Preparation of the Filtering Device

Inspect the filtering device (filtering tube, filtering cap, bottom attachment with Millipore-gridded membrane) for level of cleanliness (all parts of the filtering device that have been exposed to previous

samples should be cleaned before use). If there are any deposits on the device, clean with 10-percent hydrochloric acid solution to remove all possible carbonate contaminants. Be sure O-rings are present between the filtering tube and the bottom attachment. Transport the filter paper from packaging to the clean filtering-device using tweezers, avoiding contact with skin.

Secure the filter paper on to the device by wetting the bottom attachment of the device using DIW and centering the filter paper in position. The water should saturate the filter paper and hold it in place during assembly. Fit and screw the two lower parts of the filtering device together, sandwiching the filter paper. Use the two fitted wrenches provided with the filtering device to tighten the two parts together to avoid leaks.

Filtering

1. Check the pH of the sample to be filtered using litmus paper, minimizing the amount of time the sample is exposed to air. If the pH is <10, do not filter that sample. Be sure to replace the cap tightly until you can add more ammoniacal $SrCl_2$ to precipitate the remainder of the sample.
2. Saturate the filter tube with nitrogen using the filter cap that is connected to the tank of compressed nitrogen. Open the nitrogen tank and set the regulator to a value not to exceed 15 pound-force/square inch (psi).
3. Turn on the peristaltic pump and begin pouring (decanting) the sample into the opening at the top of the filtering device. Filtered liquid should already be pouring into the flask. This liquid should be clear in appearance. The appearance of a whitish, cloudy liquid indicates a broken or improperly positioned filter paper. If this occurs, see "Filtering Troubleshooting."
4. Screw on the cap of the filtering device, allowing nitrogen to flow freely into the filtering tube. Adjust the pressure to achieve an ideal flow of sample without forcing it through the seams of the filtering device (pressure should not exceed 15 psi).
5. Turn off nitrogen pressure once liquid in the filtering device has passed through the filter.
6. Pour the remaining sample into the filtering tube, add DIW to the sample bottle, and swirl to collect remaining precipitate. Add more water and swirl five or more times to collect all precipitate and continue filtering.
7. Add a small amount of DIW to the filtering device and reattach its cap. Turn on nitrogen pressure and allow water to pass through sample and filter paper, neutralizing the precipitate. Continue until ≥5 L of water have flushed through the filter. When the flask of flow-through water is full, separate it from filtering device, turn off and remove the peristaltic pump, and pour flow-through water directly into a sink. Reconnect the filtering device, peristaltic pump, and flask and continue filtering.
8. Repeat step 7 until the precipitate is neutral (pH is between 7 and 8). Test the precipitate with litmus paper by turning off the peristaltic pump and by first testing the flow-through water. If this water is not neutral, do not test the precipitate. The sample may need up to 10 L DIW to pass through before yielding a neutral pH result. If the flow-through water is neutral, turn down the pressure on the nitrogen regulator and use the fitted wrenches to unscrew the top and bottom portions of the filtering device, exposing the filter paper. The precipitate can be tested for its pH value using litmus paper.

Baking Sample and Filtering Device Cleanup

1. After the pH of the precipitate is neutral, use a small spatula to transfer the filter paper from the filtering device to a clean glass Petri dish with a sample ID label.
2. Place the Petri dish into a 90 °C oven for heating overnight. More time in the oven is acceptable.
3. Discard the liquid from the flask and rinse the flask and stopper with DIW.
4. Clean both halves of the filtering device with 10-percent hydrochloric acid and then with DIW. Rinse off all acid because remnant hydrochloric acid will dissolve subsequent carbonate precipitates.

5. Close the nitrogen tank.

Filtering Troubleshooting

1. Filtering device leaks under pressure: Relieve nitrogen pressure and allow any liquid left in the filtering device to drain into the flask. Turn off the peristaltic pump and remove the flask from the filtering device. Disassemble the filtering device and check for the cause of the leak. Pay special attention to the Teflon seal that is in direct contact with the filter paper; the Teflon ring may need to be replaced. Remove the filter paper from the tray. Place a new piece of filter paper on the filter tray portion of the filtering device and place the older leaky filter paper on top of it. This will act as a cushion and help close the gap that might be the cause of the leak. Reassemble the filtering device and filter the remainder of the sample.
2. Broken or improperly seated filter paper: If a filter paper breaks or becomes seated improperly while filtering, the sample will bypass the filter and will flow directly into the flask. When this happens, first allow all the liquid in the filtering device to drain into the flask. Remove the filtering device from the stopper and place it in an upright position. Disassemble the filtering device very carefully to avoid spilling any precipitate that may be trapped inside. Use a spatula or tweezers to lift the paper from the filter tray and place it in its proper position. If the filter paper is broken, place a funnel in the mouth of the flask and hold the broken filter over it with tweezers. Rinse all the precipitate from the filter paper into the flask and refilter the solution.
3. The pH or amount of sample requires >5 L of water to neutralize: Samples with substantial amounts of precipitate can also be problematic. While filtering and neutralizing the $SrCO_3$ precipitate, the precipitate may form a very thick layer on the surface of the filter paper and become impossible to filter effectively. This is indicated by the presence of a substantial amount of precipitate in the water sample and by the fast movement of water through the filter system with little water passing through the precipitate during filtering. When this occurs, the collected sample and filter paper are removed from the filtering system, placed in a jar filled with DIW, and vigorously agitated until the sample precipitate breaks into finer particles. This process increases the surface area of sample precipitate relative to the water. The damaged filter paper is discarded, a new one is placed on the filter system, the contents from the jar are then filtered, and the neutralizing/filtering continues per regular protocol.

Transferring Sample from Filter Paper to Vial

1. Transfer the sample from filter paper into a clean 3-inch (250-μm) diameter sieve.
2. Shake the sieve from side to side to facilitate the sample passing through the sieve to homogenize it. Larger pieces of sample might require the use of a spatula or glass pestle to help break the DIC sample into smaller pieces, allowing it to pass through the sieve.
3. Tap the mesh and sieve to disengage any remaining product that could be stuck to the mesh.
4. Pour the sample from the sieve receiving pan back onto the weigh paper and fold it to easily transfer the sample into an air-tight glass vial labeled with sample identification.
5. Clean the sieve by rinsing it with 10-percent hydrochloric acid and by running DIW through the sieve to remove any hydrochloric acid.
6. The sieve should be completely dry before homogenizing another sample.
7. Drying the sieve can be ensured by applying acetone to all inner surfaces of the sieve and dish and then putting them in a 90 °C oven.
8. The sample is now ready to be processed using the protocol in appendix F (“Step-by-Step Procedure to Evolve and Extract CO_2 from Carbonates”).

Appendix D. Carbonate Sample Record Sheet

Carbonate Sample Record Sheet

Mar-08

C#	Aliquot #	Aliquot Sample Weight	Date Reacted	Reaction Temperature (°C)	Date Extracted	Pentamanifold		CO_2 Yield
						Port #	Reading (μmol)	
C-								
Notes:								
C-								
Notes:								
C-								
Notes:								
C-								
Notes:								
C-								
Notes:								
C-								
Notes:								
C-								
Notes:								

Appendix E. Procedure for Phosphoric Acid (H_3PO_4) Preparation

Nearly 100-percent H_3PO_4 with a specific gravity >1.92 g/cm^3 is prepared in this procedure from PURANAL™ grade orthophosphoric acid (minimum 85 percent) and Puriss grade P_2O_5 or trade names of equivalent purity. The P_2O_5 is used to increase the purity of the H_3PO_4 by binding with water and supersaturating the H_3PO_4 solution.

Materials

- 85-percent H_3PO_4 (2,500 mL)
- 30-percent H_2O_2 (5 mL)
- P_2O_5 (2 kg)
- CrO_3 (5–100 mg)

Notes

- Gloves and a face mask must be worn at all times while handling P_2O_5.
- A useful thermometer/stirring rod can be devised by enclosing a thermometer in a large piece of heavy-walled Pyrex tubing with the bottom sealed off to prevent the acid from touching the thermometer. Otherwise, the ink on the thermometer will be liberated by the hot H_3PO_4.
- During the final cooling stage, the beaker is kept covered with plastic wrap between additions of P_2O_5.

Procedure

1. Exactly 2.5 L of H_3PO_4 is poured into a 5-L beaker on a magnetic hotplate/stirrer inside a fume hood. Use a polytetrafluoroethylene spin bar.
2. Slowly add the P_2O_5. Care must be taken during the initial stage of P_2O_5 addition because the reaction can be vigorous when the powder contacts the wet acid.
3. It normally takes approximately 2 kg of P_2O_5 to obtain the required, final specific gravity of >1.92 g/cm^3. This quantity of P_2O_5 is gradually added over a period of 2 to 3 hours with constant stirring and heating to a temperature of approximately 80 to 100 °C. (The powder initially forms gelatinous lumps, which will gradually dissolve). To minimize absorption of atmospheric moisture, the beaker is covered with a sheet of Viton.
4. During the heating process, slowly add the 30-percent hydrogen peroxide (H_2O_2), which is used to oxidize organic impurities.
5. A few crystals of CrO_3 (~50 mg) are added to oxidize organic material or other possible impurities from the starting acid solution. These crystals are added at the final dissolution stage and the heating and stirring are continued until all the P_2O_5 has dissolved.
6. The complete process takes 4 to 5 hours.
7. The stirrer hotplate is switched off, and the acid covered with the Viton sheet is cooled to room temperature before checking the specific gravity. If the specific gravity is <1.92 g/cm^3, the acid must be reheated and more P_2O_5 added.
8. The final volume of the acid should be approximately 3 L. Cool and transfer to glass bottle(s). Use Polyseal caps on bottles to keep moisture out and store in an acid-safe cabinet until required for use. It is best if the acid is aged for at least 1 month before use.

Appendix F. Step-by-Step Procedure to Evolve and Extract CO_2 from Carbonates

Reaction with H_3PO_4

1. Turn on the sample preparation line diffusion pump and fill dewar with LN_2.
2. Weigh out the appropriate amount of $SrCO_3$ sample to yield 100 to 200 μmol CO_2. If the sample concentration is unknown, use 20 to 35 mg of sample and add it to the main section of the reaction vessel. If the amount of sample is very small, consult supervisor.
3. Add 2 mL 100-percent H_3PO_4 to the sidearm of each reaction vessel.
4. Grease ground-glass joint of carbonate top with Apiezon N stopcock grease and make sure the stopcock is open.
5. Put carbonate tops on reaction vessel bottoms. Place a rubber band over the top of the carbonate vessel and underneath the arm to position the assembly properly. Do not rotate joint.
6. Repeat for remaining samples.
7. Close stopcocks to the diffusion pump and rough pump.
8. Wipe the vessel tops with a lab tissue and grease tops. Put carbonate reaction vessels on the preparation line. Secure reaction vessels to the evacuation line with a rubber band.
9. Open the stopcock to rough pump. Check that the U-tube stopcock is open.
10. Open all stopcocks just above the reaction vessels, now open to rough pump.
11. After 30 seconds, rotate all joints to spread stopcock grease.
12. Close the rough pump and open the diffusion pump.
13. After several minutes, check that the stopcock on the thermocouple gauge is open.
14. After good vacuum has been obtained (1–3 hours), close reaction-vessel stopcocks.
15. Close stopcocks just above reaction vessels, closing the line's exposure to the atmosphere.
16. Close stopcock to diffusion pump.
17. Close stopcock to thermocouple gauge (optional).
18. Remove all sample-reaction vessels, wiping grease clean with lab tissues.
19. When ready to react samples, tip reaction vessel to a 15º angle for 10 seconds to allow acid to move into the main arm of vessel.
20. Allow the sample to sit upright at room temperature for 24 hours. Note any unusual reactions.

Extracting CO_2 from Carbonates and Preparing for Extraction

1. Turn on the pentamanifold diffusion pump and fill dewar with LN_2.
2. Grease ground-glass joint of carbonate-reaction vessels Apiezon N stopcock grease.
3. Place reaction vessels on the pentamanifold.
4. Close stopcocks on thermocouple gauges (optional).
5. Close stopcock to diffusion pump.
6. Open stopcock to rough pump.
7. Open top pentamanifold stopcocks.
8. Open stopcocks on upper fingers.
9. Open bottom pentamanifold stopcocks.
10. Rotate vessels to seal.
11. After 30 seconds, close stopcock to rough pump.
12. Open stopcock to diffusion pump.
13. Check second stopcock in line to diffusion pump to make sure it is open.
14. Prepare thin, dry ice slush (crushed dry ice and isopropanol) into a small dewar and set aside.

15. After 2 minutes, be sure all stopcocks on thermocouple gauges are open.
16. Wait until thermocouple gauges indicate good vacuum; this should take <5 minutes.

Transfer Sample to Lower Fingers

1. Close top pentamanifold stopcocks.
2. Close stopcocks to thermocouple gauges (optional).
3. Put small LN_2 dewar on lower fingers using aluminum mesh only under dewar.
4. Open stopcocks on reaction vessels to freeze samples into lower fingers.
5. Transfer sample labels to upper pentamanifold.
6. Wait 3 to 5 minutes.
7. Open stopcocks to thermocouple gauges.
8. After thermocouple gauges have stabilized, top off dewar with LN_2 and wait 30 seconds.
9. Close bottom pentamanifold stopcocks.
10. If any thermocouple gauge reads above 1 millivolt, the respective sample-reaction vessel has leaked. In such a case, close the upper pentamanifold stopcock for that section and discontinue processing that sample. Mark both the top and bottom of carbonate reaction vessel with tape and an appropriate message that each needs to be degreased and tested further.
11. Evacuate noncondensables quickly by opening top stopcocks for <30 seconds. Gauges should move to the right to point of ultimate vacuum.
12. Close the top pentamanifold stopcocks.

Transfer Sample to Upper Fingers

1. Transfer LN_2 dewar from lower fingers to upper fingers.
2. Put dry ice slush on lower fingers using wooden block and aluminum mesh under dewar. This keeps water frozen in lower fingers while allowing the CO_2 to be transferred.
3. Remove carbonate reaction vessels.
4. Put on clean gas sample tubes that have been greased. Do not rotate. Secure with a rubber band.
5. Wait until needles on the thermocouple gauges have gone all the way back to the right.
6. Top off dewar with LN_2 and wait 30 seconds.
7. Open top pentamanifold stopcocks to evacuate any noncondensable gases.
8. After 10 seconds, close stopcocks on upper sample fingers.
9. Close stopcocks on thermocouple gauges (optional).
10. Close the stopcock to the diffusion pump.
11. Open the stopcock to the rough pump.
12. Remove dry ice slush.
13. Replace with warm water over fingers, helping to heat and pump away water that may have been trapped in the fingers.
14. Open bottom pentamanifold stopcocks to evacuate air above sample tubes.
15. Rotate gas sample tubes to seal.
16. After 30 seconds, close stopcock to rough pump.
17. Open stopcock to diffusion pump.

Check Yield of Samples in Upper Fingers

1. Remove LN_2 dewar from upper fingers and monitor pressure sensors to make sure they do not go above a meter reading of 280; otherwise, an alarm will sound. If an alarm does sound, replace LN_2 dewar, notify supervisor, and see special procedures for large samples.
2. Warm upper fingers to ambient temperature (approximately 25 °C) with a beaker of water.
3. Record on data sheet the readings of upper finger gauges for each sample; these are the CO_2 yields in micromoles.
4. Remove beaker of water from upper fingers.
5. Refreeze samples on upper fingers with LN_2 using only aluminum mesh under dewar.

Check Gas Sample Tubes for Good Vacuum

1. Open thermocouple gauge stopcocks.
2. Wait until thermocouple gauges have reached ultimate vacuum. Needles on gauges should be all the way to the right.
3. Close all top pentamanifold stopcocks.
4. Open all glass stopcocks on gas sample tubes.
5. If any thermocouple gauge drops below 7 millivolts, then do the following:
   a. Close the bottom pentamanifold stopcock to each leaking sample vessel.
   b. Remove leaking sample vessel, mark it for regreasing, and place it in the hood.
   c. Put on another clean glass sample tube; do not rotate.
   d. Close stopcock to diffusion pump.
   e. Open stopcock to rough pump.
   f. Open top pentamanifold stopcock.
   g. Open bottom pentamanifold stopcock.
   h. Rotate glass sample tube.
   i. After 15 seconds, close the stopcock to rough pump.
   j. Open stopcock to diffusion pump.
   k. Wait until thermocouple gauge reaches ultimate vacuum.
   l. Close top pentamanifold stopcock.
   m. Open glass stopcock on gas sample tube.
   n. If gas sample tube has leaked (<7 millivolts on the thermocouple gauge), repeat procedures *a* through *m.*
6. Open all top pentamanifold stopcocks to evacuate noncondensables.

Transfer Samples to Gas Sample Tubes

1. Close all top pentamanifold stopcocks.
2. Transfer sample labels to gas sample tubes.
3. Arrange LN_2 under gas sample tubes, approximately 1 inch into LN_2.
4. Open upper finger stopcocks.
5. Remove LN_2.
6. Put dry ice slush on upper sample fingers using aluminum. Be sure the level of the slush dewar is higher than that of the LN_2 in dewar that was just removed.
7. Wait until pressure stabilizes on thermocouple gauges.
8. Raise the level of LN_2.
9. Open top pentamanifold stopcocks to evacuate noncondensable gases.
10. After 10 seconds, close glass stopcocks of gas sample tubes.

11. Close bottom pentamanifold stopcocks.
12. Close thermocouple gauge stopcocks.
13. Remove dry ice slush.
14. Put beaker of warm water on upper fingers.
15. Remove LN_2 dewars.
16. Remove gas sample tubes that are now ready for isotopic analysis.
17. Wipe grease clean and regrease for placement on CO_2 gas line for analysis. Secure with a rubber band.
18. Record placement of samples by appropriately filling out the DuPont Sample Record Sheet (appendix H) with a pen and updating the headings in the DuPont computer for proper LIMS retrieval of information.
19. Remove the beaker of water from upper fingers.
20. Proceed with next set of five carbonate reaction vessels. Go immediately to step 2.
21. When finished working with samples, close stopcock to diffusion pump, turn off diffusion pump, and remove large LN_2 dewar.

Special Procedure for Large Samples

1. When the alarm goes off due to large sample size, check gauges and make note of which sample or samples are too large.
2. Refreeze samples in the upper fingers with LN_2.
3. Make a note in the sample book that samples were too large to measure and were expanded into system.
4. Check gas sample tubes (of ports with large samples) for good vacuum. Leave stopcocks open.
5. Only on ports with large samples:
   a. Close thermocouple stopcocks.
   b. Close top stopcocks.
   c. Close first-tier pentamanifold stopcocks.
   d. Open sample fingers.
6. Remove LN_2 on upper sample fingers.
7. Monitor movement of CO_2 on gauges. After several minutes, if reading on the pressure sensor is constant and at a level that can be analyzed (>20), close stopcocks to upper fingers.
8. Open stopcocks to thermocouple gauge, top stopcocks, and first-tier manifold stopcocks of large samples.
9. Continue with “Check Yield in Upper Fingers.”

Cleaning Gas Sample Tubes

1. Turn on sample preparation line diffusion pump and fill dewar with LN_2.
2. Wipe excess grease off ground glass-joint of gas sample tube, open stopcock to air, inspect glass joint for streaking, regrease glass joint, and place dirty sample tube on cleaning line without rotating joint.
3. Close air inlet stopcock.
4. Slowly open stopcock to diffusion pump.
5. Rotate tubes to seal joints.
6. Lower heaters over gas sample tubes; go very slowly near the bottom.
7. Check that heaters are seated properly over tubes.
8. Turn Variac autotransformer on and set to 70.
9. Turn timer to 60 minutes. Set laboratory timer to 1 hour to remind you to close sample tube stopcocks when heating is finished. Close diffusion pump stopcock and turn off pump if done for the day.

Appendix G. CO_2 Mass Spectrometer Checklist

Check the following items and then indicate this on the following chart:*

1. Reference gas loaded as necessary.
2. Enough standard gas for the night.
3. Piston Hoke valves open/sidearm closed.
4. All four piston lights are on.
5. Piston N_2 pressure ~3 psi.
6. N_2 tanks: Valve tank P >1000 psi.
   Piston tank P >200 psi.
7. Printer paper; clean ink cartridge.
8. Minor peak centered and HV scan focused on 280.
9. Wash used vessels and put in oven.
10. CO_2 line toggle valve closed.
11. Toggle valves on slush trap open.
12. Computer control button on red.
13. Water vessels shaking.
14. Equilibration line temp = 25 ± 0.1 °C.
15. Dry ice slush replenished at end of day.
16. All valves are on auto (especially 91, 93, and 98).
17. Open gas sample stopcocks; be sure the air between the auto valve and the sample stopcocks was previously evacuated.
18. Stay to watch first gas sample run.

Mon	Tues	Wed	Thurs	Fri	Sat	Sun

*Steps 9 to 15 refer to water equilibration line.

Appendix H. DuPont Sample Record Sheet

DuPont Sample Record Sheet

SPECIAL COMMENTS:

DATE _____

ANALYST _____

TIME CO_2 ADDED _____
[when water equilibration line is also running]

CARBONATES

PORT #	SAMPLE ID	O	C	O + C	COMMENTS
70					
71					
72					
73					
74					
75					
76					
77					
78					
80					
81					
82					
83					
84					
85					
86					
87					
88					
89					

Appendix I. Step-by-Step Procedure to Determine and Apply Correction Factors

1. Open “Correction Factors and Normalization Equations” in LIMS-LSI.
2. Select the DuPont DI-IRMS (D) and element carbon.
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. Go back to LIMS-LSI main menu by closing open windows.
10. Choose “Print Samples in Progress.”
    a. Open “Sample in Progress.”
    b. Choose appropriate isotope.
    c. Choose appropriate prefix (“C” for Carbonate).
    d. Put in sample ID range from “Sample to Be Analyzed” sheet. Click Print.
11. Review results; determine samples that need to be repeated.

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

1. Open “Store Samples in Progress” in LIMS-LSI.
2. Choose appropriate isotope (“C” for Carbon).
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 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 that are related to this project.

Appendix K. DIC Results Report

The Reston Stable Isotope Laboratory has analyzed the following samples received from you for isotopic analysis. For questions, please contact by e-mail at isotopes@usgs.gov or Haiping Qi at voice 703–648–6338 or fax 703–648–5274. Please refer to Our Lab ID. These isotopic results supersede any results that may have been previously submitted to you.

Method

Carbonate is reacted with 100-percent phosphoric acid at 25 ºC to liberate carbon dioxide, which is collected, purified by vacuum sublimation, and analyzed on a mass spectrometer (McCrea, 1950).

Reporting of Relative Carbon Isotope Ratios

Effective April 18, 2006, relative carbon isotopic results are reported in per mil relative to VPDB and normalized (Coplen and others, 2006) on a scale such that the relative carbon isotope ratios of L-SVEC Li_2CO_3 and NBS 19 $CaCO_3$ are –46.6 and +1.95 ‰, respectively.

The carbon isotopic compositions of carbon-bearing, internationally distributed isotopic reference materials, had they been analyzed in this laboratory with your samples, are as follows:

NBS 19	Calcium carbonate	+1.95 (exactly)
NBS 18	Calcium carbonate	–5.01
IAEA-CO-1	Calcium carbonate	+2.49
IAEA-CO-8	Calcium carbonate	–5.76
L-SVEC	Lithium carbonate	–46.6 (exactly)
IAEA-CO-9	Barium carbonate	–47.32

USGS24	Graphite	–16.05
NBS 22	Oil	–30.03
IAEA-CH-3	Cellulose	–24.72
IAEA-CH-6	Sucrose	–10.45
IAEA-CH-7	Polyethylene	–32.15
IAEA-600	Caffeine	–27.77
IAEA-601	Benzoic acid	–28.81

USGS40	Glutamic acid	–26.39
USGS41	Glutamic acid	+37.63

RM8562	Carbon dioxide	–3.72
RM8563	Carbon dioxide	–41.59
RM8564	Carbon dioxide	–10.45

The expanded uncertainty ($U = 2\mu_c$) obtained from long-term $\delta^{13}C$ results is ±0.2 ‰, unless otherwise indicated. This means that if the same sample were resubmitted for isotopic analysis, the newly measured value would lie within the uncertainty bounds 95 percent of the time (Coplen and others, 2006).