



COLLECTION AND MEASUREMENT OF CARBON ISOTOPES IN SEAWATER DIC

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1. INTRODUCTION

The stable and radio-carbon isotopic content of seawater dissolved inorganic carbon (DI¹³C and DI¹⁴C, respectively) is measured by extracting the inorganic carbon as CO₂ gas. A small portion of the gas is analyzed on a stable isotope ratio mass spectrometer and most of the gas is converted to graphite and inserted into an accelerator mass spectrometer where the number of ¹⁴C atoms are counted. The sampling procedure described below is straightforward, but it is important to stress the need for clean, careful sampling techniques to ensure an uncompromised sample. In order to minimize exchange with atmospheric CO₂, sample transfers must be as rapid as possible. The use of an accelerator mass spectrometer to measure ¹⁴C in seawater samples has greatly reduced the size of the sample required for the measurement, but it has also greatly increased the importance of collecting the sample in a clean, ¹⁴C-free, environment. Contamination of the sample container can arise from collecting and handling the sample on a contaminated surface and from exchange of the CO₂ in the sample with atmospheric CO₂. Radiocarbon is used in the laboratory and at sea to measure oceanic productivity and inadvertent spills can leave isolated spots that are severely contaminated. The levels typically used in tracer experiments can be several million times modern levels and very small residual amounts can ruin the measurement of natural levels of ¹⁴C. In order to avoid contamination from this source, we recommend that surfaces where the samples are collected or handled be covered with fresh disposable sheets of plastic and that disposable gloves (changed often) be worn during sampling. The pre-cruise preparations should not be performed in a laboratory in which ¹⁴C has been used as a spike. Further details about how to avoid inadvertent contamination can be found at <http://nosams.whoi.edu/clients/handling.html>.

2. BOTTLE CLEANING PROCEDURE

The bottle used for the collection of seawater is shown in Figure 1; it is a 500 ml Pyrex (or Pyrex-equivalent) reagent bottle with a 29/26 standard taper ground glass joint and a solid stopper. Lab Glass is the best supplier of this bottle and will provide it precleaned.

To clean the bottles, first wipe any excess grease from the stopper and ground glass joint on the bottle. In a hood, further clean the stopper and joint with laboratory wipes soaked in xylenes and acetone to prevent transfer of grease from the stopper region to the inside of the bottle. After washing with solvents, allow the pieces to dry in a well-ventilated area. When using xylene, solvent-impermeable gloves should be worn. The above steps are not necessary for bottles fresh from the factory.

The following steps are necessary for all bottles, unless an arrangement has been made with the factory to provide bottles cleaned in the same manner. When the labels and grease have been removed and solvents have evaporated, wash the bottles and stoppers with a dilute soap solution, rinse well with warm tap water, rinse the bottles and stoppers with 10% HCl, and finally rinse three times with distilled water. From this point on, do not leave bottles upright without covering the opening with clean aluminum foil. The foil can be rinsed with distilled water. Bake the glassware overnight in a 450° C oven. When the bottles and stoppers are dry and cool, place tape or laboratory wipe in the ground glass joint of each bottle. Part of the tape should extend over the lip of the joint. The tape or wipe is useful for preventing the stopper from seizing when shipping the bottles. Prior to packing for use at sea, the bottle must be cleaned. Finally, place the stopper in the bottle.

After cleaning and capping, each bottle must have a label affixed. The bottles used for the CLIVAR Repeat Hydrography Program each have a unique identifying number and do not require a label. If using an unmarked bottle, spaces for the following information are suggested for the label:

- Sample number or ID
- Sample location
- Cruise name and number, Leg number, Station/cast number (if collected at sea)
- Depth
- Date
- Time

3. SHIPPING PREPARATION

When the bottles have been washed, dried, and labeled, weigh the bottle plus stopper and record the weight. Bottles used for the CLIVAR Repeat Hydrography Program have pre-recorded weights and do not require this step. Place them in a packing crate for shipping. Place a rubber band (5”L x 5/8”W) on each bottle. The bottles should fit snugly in the crate and the crate should be sealed securely before shipping; each crate holds 16 bottles. In order to reduce the possibility of contamination during shipping and storage, the crates should be covered with a disposable plastic bag, which is closed with a reusable tie.

The items listed in Table 1 must be prepared before collection. The easiest and perhaps the safest way to prepare saturated HgCl₂ solutions for use at sea or in the field is to pre-weigh the HgCl₂ powder (ACS grade, crystal) into plastic bottles and add distilled water at sea.¹ The solubility of HgCl₂ is approximately 7g/100cc at 20°C; each sample requires 100 µl of solution. Thus, collection of 1000 water samples would require only 100 ml total solution.

Table 1: Items to be prepared for shipping with sample bottles.

1. Saturated aqueous HgCl₂ solution (see footnote 1)
2. 100 µl Eppendorf pipette with yellow tips
3. Plastic pipette with bulb, a cooking baster is ideal.

¹ The Merck index lists HgCl₂ as a “violent poison” for which 1 or 2 g is frequently fatal. After using HgCl₂ (either as a powder or in solution), the user should always wash thoroughly before eating or drinking. The powder should not be inhaled because it is corrosive to mucous membranes.

4. Swabbing tool (a stick with laboratory wipes attached)
5. Tygon drawing tube (pre-treat by soaking in clean seawater for at least one day)
6. Tubes of Apiezon “L”, “M”, or “N” grease
7. Rubber bands (5”L x 5/8”W)
8. Laboratory wipes
9. Teflon tape
10. Labels

4. SAMPLING

A. Bottle Preparation Procedure

The integrity of $DI^{13}C$ and $DI^{14}C$ samples can only be guaranteed if the samples are collected using the proper procedures and collected in ^{14}C -free environment. The bottles should be handled as little as possible and removed from their packing crates only when necessary. A data sheet(s) should be kept for each crate of bottles. Information regarding the history of each crate of bottles should be recorded on this sheet. This information should include identification of the laboratory in which the bottles were prepared, the shipping and storage history (dates and location) of each crate, information regarding the condition of laboratories and storage facilities (e.g., refrigerated or not) and identify other sampling programs in progress on the ship.

B. Pre-sampling procedure

Clean, disposable gloves should be worn any time the bottles are handled. When the bottles are removed from the crates, they should not be placed in direct contact with any surface on the ship either on deck or in the laboratory. Plastic sheets or garbage bags can be placed on any surfaces the bottles must touch. Bottles for each cast should be transferred from their packing crate to the plastic sample holder designed for use during transport and filling of the bottles. Prior to actually sampling the seawater, as much information as possible should be written on the bottle label. This information may also be entered onto a data sheet (Figure 1).

The data sheets should be used to record information regarding sea state at the time of sampling, other programs sampling simultaneously or sequentially from the water sampler, and any comments regarding unusual conditions.

C. Sample Transfer Procedure

The procedures described here are based on those used for the collection of DIC samples and assume that samples will be collected from a Niskin bottle. When the bottles have been readied for sampling, check to be sure that all the items in Table 1 are on hand and then proceed to collect the seawater samples.

Immediately prior to sampling, remove the glass stopper and the laboratory wipe (always make sure the strips of lab wipe or Teflon tape have been removed before collecting any seawater). Place the tygon tubing on the Niskin bottle and flush with approx. 50 ml of water. Then place the tubing inside the sample bottle, making sure the tube reaches to the bottom of the bottle and the vent at the top of the Niskin bottle is open. Fill the bottle with approximately 50 ml of water; gently swirl around to rinse the sides of bottles and discard; repeat. With the tygon sampling tube at the bottom

of the bottle, fill with enough water to fill the bottle 1.5 times; this can be accomplished by observing the amount of time it takes to fill the bottle and allowing the bottle to overflow for half this time, stopper the bottle with an ungreased stopper and fill the remainder of the bottles from the cast. If two samplers are available, have one sampling from the niskin and one in the lab poisoning/stoppering the bottles. That way you don't have to stopper the bottle twice. Using this procedure, an AMS water sample will require approximately 850 ml of water.

When the bottle has been filled, remove it to a safe, dry place and continue preparing the sample for storage in the following manner. Remove the stopper; wipe clean and dry; using the grease syringe, apply a thin layer of grease in a wavy pattern around the stopper (Figure 2); set the stopper aside. Apiezon-M grease should be brought along for use. Using the large pipette or just by pouring, remove enough water for a 5-10 ml headspace to exist in the bottle; this level can be marked on the bottle (Figure 3). Using the Eppendorf pipette, add 100 μ l of the saturated HgCl_2 solution to the bottle (Figure 4). Carefully and completely wipe the inside of the ground glass joint dry using lab wipes and place the stopper in the bottle. Care must be taken not to put your finger in the sample. **The joint MUST be dry for the grease seal to work properly!** Twist the stopper around while applying pressure to ensure that a good seal is made (Figure 5). There should be no air streaks within the greased neck of the bottle. Secure the bottle top with one rubber band placed over the entire bottle. If a duplicate sample is to be taken, start filling the second bottle immediately using the same procedure. After both bottles are filled, capped, and secured with an elastic band, shake gently to mix poison in.

D. Sample Storage Procedures

After all samples from one cast have been taken and sealed, each label/data sheet should be checked to make sure it contains the necessary information, and the integrity of the greased seals should be checked. Where appropriate, data from these samples should be entered into a database or spreadsheet. Necessary information to include are: station number, latitude, longitude, sampling date and time, depth to bottom, sample bottle type (e.g., rosette), cast number, rosettes bottle number, sample depth or pressure, AMS bottle number, and AMS box number. When the data for all the samples have been recorded properly, the samples should be transferred to the shipping crate. The crate should be closed, secured with tie wraps and stored in a temperature-controlled environment (i.e. the ship's science hold). Seawater samples must not be exposed to extremes of temperature. The samples do not need to be refrigerated. **NEVER FREEZE THE SAMPLES.** If the samples are frozen, the water will expand and either dislodge the cap or break the bottle. If the sample is stored at too high a temperature, the grease will melt and run into the sample, and the sample may expand enough to dislodge the cap. According to a manufacturer's bulletin (Biddle Instruments #43C) the optimum working temperatures for Apiezon greases L, M, and N are 15-25°C. To best maintain their integrity, samples should be stored and shipped in a van which is capable of maintaining the temperature within this range.

5. LABORATORY PREPARATION OF SAMPLES

In this section, we discuss the methods used at the NOSAMS and UW laboratories to convert the inorganic carbon in seawater to the carbon target used in the accelerator. Many of the processes are automated and/or controlled through robotics, but we will not discuss the details of the automation or robotics in this document.

5.1. Logging Samples into Database

Samples shipped to the AMS facility are logged into our database upon arrival. We are careful to proceed under the assumption that any crate may be contaminated with ^{14}C . When present, disposable plastic bags are removed and discarded before transferring any crate into the AMS building. The enclosed information sheets on each crate are read and then samples are removed from their packing crates prior to transfer into the sample preparation laboratory. When the sample enters the preparation laboratory, it is assigned an AMS bar-coded sample number and data related to the processing of each sample is recorded and stored in the NOSAMS relational database. The integrity of the greased seal on each bottle is checked as the sample is logged in.

5.2. Laboratory Extraction of CO_2

Graphite targets for ^{14}C analysis are prepared from seawater in 2 steps; first, CO_2 is stripped from acidified seawater and, second, CO_2 is reduced to filamentous carbon, commonly referred to as graphite. In this section, we summarize the procedures used to extract CO_2 from seawater; detailed procedures for each of the steps at NOSAMS are on file at the AMS facility and are described in McNichol et al., 1992 and 1994.

5.2.1 Preparation of the extraction line

Before working with any samples, the vacuum extraction lines are prepared for sample stripping. The vacuum line must be pumped down and free of leaks in all regions. Stripping probes are placed on samples in a N_2 -filled glove bag; the probes are designed to mate with the 29/26 standard taper joint of the sample bottle. When the probe is on the bottle, it is transferred to the vacuum line. When the line is leak-tight, the sample stripping region of the vacuum extraction line is filled with clean CO_2 -free N_2 gas to a pressure of approximately 0.8 atm.

5.2.2 Stripping CO_2 from the seawater sample

Two methods are used to extract CO_2 from seawater samples. The first procedure is followed at NOSAMS. The cold traps for water and CO_2 are at -80°C and -190°C , respectively. When all the valves needed to recirculate the sample are open, 4 ml of 85% H_3PO_4 are added to the sample, the recirculating pump is turned on, and the sample is stripped for 10 minutes, this routinely produces >95% yield of CO_2 from the sample. After stripping is complete, the inert gas is pumped away; the extracted CO_2 is purified by removing H_2O and uncondensable gases and quantified in a known volume using a pressure transducer. The sample is then transferred to a labeled sample storage vessel. At this point the line can be prepared for another sample by pumping the line down and repeating the procedure. After all the samples on one manifold have been stripped, the bottles are removed from the line and returned to the sample crate.

At the UW, the CO_2 is extracted from seawater using a single pass procedure. The extraction line is pumped down to ~ 1 mtorr. Approximately 100ml of seawater is sucked into a pre-weighed glass bubbler. (The bubbler is reweighed after the CO_2 extraction to determine the weight of seawater in the sample.) Approximately 1 ml of phosphoric acid is added to the seawater sample to convert DIC to CO_2 . Helium is introduced into the bubbler at 40–100 cc/min flow rate. The stripping procedure is continued for ~ 20 min. Downstream of the bubbler, water vapor is removed using a trap at ~ -80

°C and further downstream CO₂ is removed using another trap at -190 °C. After the stripping is finished, the collected CO₂ is transferred to a section of the extraction line of known volume containing a pressure transducer, where the CO₂ gas pressure and temperature is measured. (The DIC concentration is determined from the measured amount of CO₂ and weight of seawater in the sample.) The CO₂ is then transferred to a vacuum flask for mass spectrometer analysis.

5.2.3 Sample Splits and Storage

The amount of CO₂ that is stripped from 500 ml of seawater (approximately 1 mmol) is much greater than that needed for producing a single target for AMS counting (5-10 times). The CO₂ samples are split into three subsamples, each of which is labeled with a bar code. One of the splits is transferred to the ¹⁴C target preparation line, another for ¹³C/¹²C analysis, and the third is archived in a flame-sealed tube. At the UW, the CO₂ sample is split into two portions, the first for immediate analysis of the ¹³C/¹²C and the second is transferred to a flame-sealed tube for archiving or ¹⁴C analysis.

5.3 Preparation of Graphite Targets for AMS

Gaseous CO₂ is converted to filamentous graphite on a dedicated vacuum line; this line has a gas transfer region and a graphite reactor region. We are currently using a modification of the method described in Vogel et al., 1987. In this method, virtually all the CO₂ is converted to filamentous graphite which forms on the catalyst (<200 mesh reduced Fe). The carbon/catalyst mixture is transferred to a target press, compressed into a target holder, and the sample is put in the queue for the AMS.

5.4 Analysis of Δ¹⁴C and δ¹³C of DIC

Radiocarbon values of WOCE Samples will be reported as Δ¹⁴C using established procedures modified for AMS applications (e.g., Stuiver and Polach, 1977; Donahue et al., 1990).

The ¹³C/¹²C of the CO₂ extracted from the seawater is measured relative to the ¹³C/¹²C a CO₂ gas standard calibrated to the PDB standard using an isotope ratio mass spectrometer (IRMS). (At NOSAMS, either a VG Prism or Optima IRMS is used at the UW a Thermo 253 IRMS is used.)

The replication of the Δ¹⁴C and δ¹³C measurements are approximately ±4.5 ‰ and ±0.03 ‰, respectively, as determined by analysis of duplicate seawater samples drawn from the same Niskin bottle.

6. ASSURANCE OF DATA QUALITY

We assure the quality of the data from this laboratory with a comprehensive program that includes:

1. Written procedures
2. Sample documentation
3. Analysis of primary standards
4. Quality assurance samples

5. Quality assurance audits

7. REFERENCES

Donahue, D.J., T. W. Linick, and A. J. T. Jull. 1990. Isotope-ratio and background corrections for accelerator mass spectrometry radiocarbon measurements. *Radiocarbon* 32, 135-142.

McNichol, A. P., A. R. Gagnon, G. A. Jones, and E. A. Osborne. 1992. Illumination of a black box: analysis of gas composition during graphite target preparation. *Radiocarbon* 34(3), 321-329.

McNichol, A. P., G. A. Jones, D. L. Hutton, and A. R. Gagnon. 1994. The rapid preparation of seawater SigmaCO₂ for radiocarbon analysis at the National Ocean Sciences AMS Facility. *Radiocarbon* 36(2), 237-246.

Vogel, J. S., D. E. Nelson, and J. R. Southon. 1987. ¹⁴C Background levels in an accelerator mass spectrometry system. *Radiocarbon* 29, 323-333.



A properly greased glass stopper before insertion into bottle.



Poison being added to sample. Make sure neck of bottle is dry BEFORE greased glass stopper is inserted.



Apply pressure downward with a clockwise rotation to get grease to “fill” the joint properly.



Joint should look “clear” with grease applied, i.e. no bubbles or wet streaks.



When filling the bottles, make sure the tygon tube is in the base of the bottle. Count how long it takes for the bottle to fill, and then double the time to allow the water to overflow the bottle. Pull out the tygon tube, and spill out enough water to leave an approx. 3/8" head space below the stopper joint. **DON'T FORGET TO ADD THE POISON!**



A properly filled and sealed water bottle.

Sample Kit #1

- 1 Baster
- 2 Zinger
- 1 Box of 12 pencils
- 2 Pair Thick rubber gloves
- 1 Roll Duct Tape
- 1 Box Large Clear plastic bags
- 1 Envelope containing 66 Nalgene labels size 2”X4”
- 1 Box of 100 tyvex envelopes
- 1 Finn pipette
- 1 Box of 39 eppendorf pipette tips
- WHOI Box Labels
- 3 tubes “M” grease
- 1 roll scotch tape
- 10 10 ml plastic syringes 5 loaded with “M” grease
- 1 10 ml plastic syringe loaded with “L” grease
- 1 25 ml vile Mercuric Chloride
- 2 60 ml vile Mercuric Chloride
- 1 ½ used tube “M” grease
- 1 Large storage bag containing roll of electric tape, MSDS (mercuric chloride),
 and vile of mercuric chloride powder
- 1 Binder containing about 100 deck log sheets
- 1 Storage bag containing pens, pencils, lab tape, markers, and electrical tape
- 2 lengths of tygon tubing (1 about 6 foot, 1 about 10 foot)
- 2 Tie down straps
- 1 Bag of vinyl gloves size M (20 gloves)
- 1 Bag of vinyl gloves size M (100 gloves)
- 1 Bag of vinyl gloves size S (100 gloves)
- 16 Shipping Labels back to WHOI
- 1 Box Zip ties
- 1 Pair Wire Snips

Sample Kit #2

- 23 Boxes large rubber bands

Sample Kit #3

- 1 Roll of shrink wrap
- 26 Boxes Kim wipes