

Measuring ^{14}C in Seawater ΣCO_2 by Accelerator Mass Spectrometry

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1. Introduction

The National Ocean Sciences Accelerator Mass Spectrometry (AMS) Facility at the Woods Hole Oceanographic Institution (WHOI) was established to provide a large number of high precision radiocarbon analyses to the oceanographic community. The facility houses an accelerator laboratory and a sample preparation laboratory that are being developed to produce a larger quantity of radiocarbon analyses annually than any other existing facility (Jones, *et al.*, 1990). The WHOI AMS system will be capable of analyzing 4300 samples/year when it is in full operation. One facet of the WOCE Hydrographic Programme is the collection of seawater samples for the measurement of $\Delta^{14}\text{C}^1$ in dissolved inorganic carbon (ΣCO_2); this program will be a major source of samples for the WHOI AMS facility.

In this document we will describe the methods used to collect and analyze seawater samples for the measurement of $\Delta^{14}\text{C}$ in ΣCO_2 . We will describe the preparations necessary prior to going to sea, the shipboard sampling and handling procedures, and the laboratory extraction procedures. The methods described here are not the only methods that can be used successfully, but are those that we have adopted at the National Ocean Sciences AMS Facility. Other methods currently in use are described in Bard *et al.* (1987) and Schlosser *et al.* (1987). Samples submitted to this facility will be handled in the manner described here and processing of samples will be facilitated by submission in the sample bottle described here.

The radiocarbon content of seawater ΣCO_2 is measured by extracting the inorganic carbon as CO_2 gas, converting the gas to graphite, and counting the number of ^{14}C atoms in the sample directly using an accelerator mass spectrometer. The sampling procedure described below is straightforward, but it is important to stress the need for clean sampling techniques. The use of an accelerator mass spectrometer to measure ^{14}C 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, ^{14}C -free, environment. Contamination of the sample container can arise from collecting and handling the sample on a contaminated surface and from exchange of the CO_2 in the sample with atmospheric CO_2 . 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 ^{14}C . 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

$^1\Delta^{14}\text{C} = \delta^{14}\text{C} - 2(\delta^{13}\text{C} + 25)(1 + \delta^{14}\text{C}/1000)$

be performed in a laboratory in which ^{14}C has been used as a spike. In order to minimize exchange with atmospheric CO_2 , sample transfers must be as rapid as possible.

Because this is a new and developing facility, this document should be considered the first of a series. The National Ocean Sciences AMS Facility is currently in the process of becoming fully operational with a program and instrumentation that is new to the community. The methods described here may be modified over time as we learn more about optimizing the sample preparation techniques for accuracy and precision of radiocarbon analyses on our machine. Any investigator wishing to use this document should check with the AMS facility to ensure that these are the most up-to-date procedures in use. We anticipate publishing an updated version of this document yearly.

2. Pre-cruise Preparation

2.1 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 pre-cleaned. Teflon tape or a piece of laboratory 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.

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. Finally, place the stopper in the bottle.

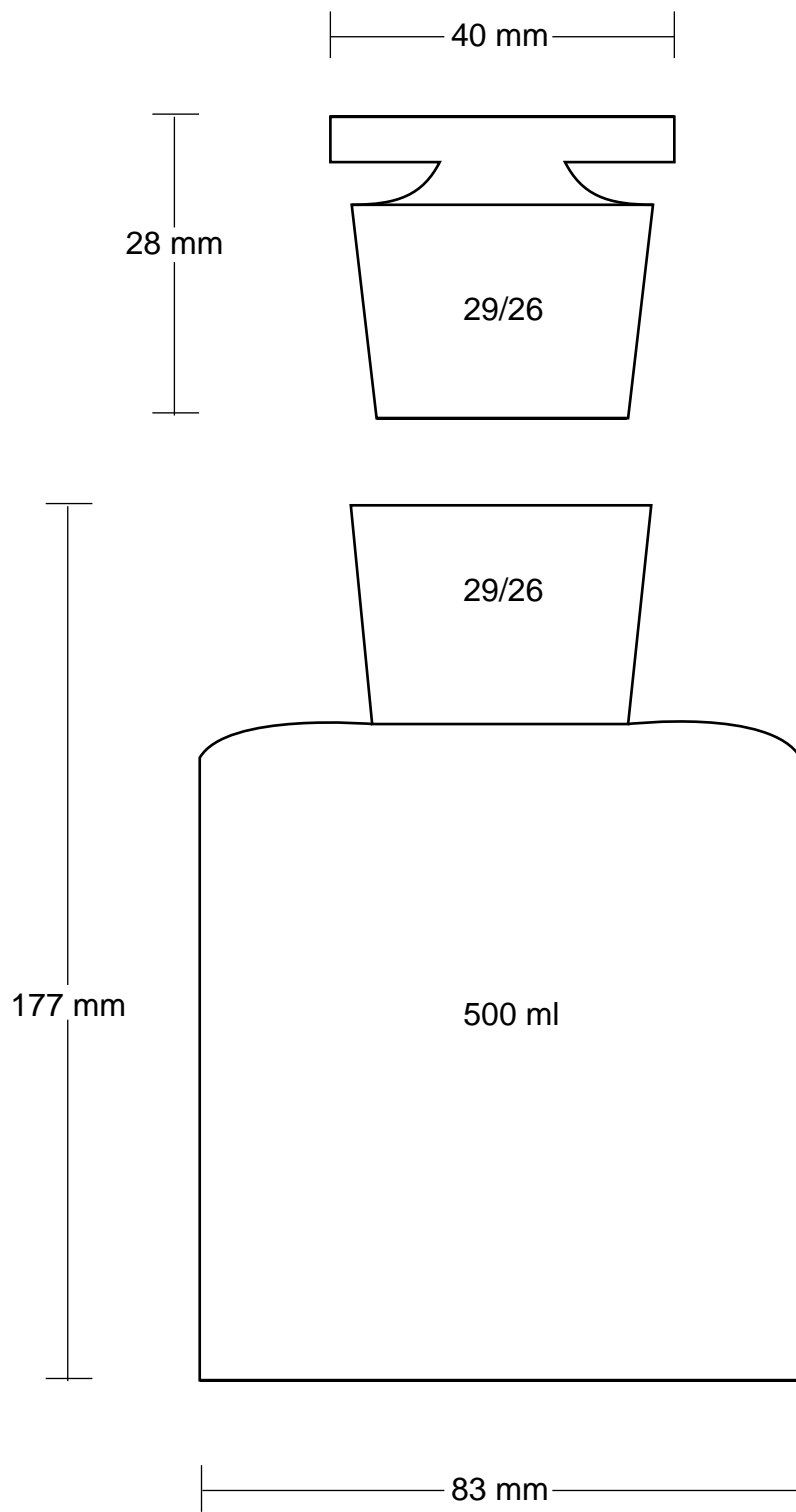


Figure 1: Sample bottle

After cleaning and capping, each bottle must have a label affixed. Spaces for the following information are contained on the label:

- Sample number
- Cruise name and number
- Leg number
- Station/cast number
- Depth
- Date
- Time

As an alternative to using a label, each bottle and stopper combination may be etched with a unique number; in this case, all the information pertaining to each bottle should be entered on a data sheet. In either case, a “chain-of-custody” form in a water-proof bag must travel with each bottle or case of bottles. This is the minimum amount of information we will need to process a water sample. If sample information is transferred to a data sheet or a database, we can accept a 1-page data sheet or 1-screen of information from a computer disk. Database information must be entered in Lotus 1-2-3 or equivalent format for us to accept it.

2.2 Shipping Preparation

When the bottles have been washed, dried, and labelled, weigh the bottle plus stopper and record the weight. Place them in packing crate for shipping. Place a plastic clip and rubber band (5” L x 5/8” W) for each bottle in the crate. Lab Glass will supply bottles with an appropriate clip. 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 going to sea. The easiest and perhaps the safest way to prepare saturated HgCl_2 solutions for use at sea is to pre-weigh the HgCl_2 powder (ACS grade, crystal) into plastic bottles and add distilled water at sea. Please note that extreme care should be taken when dealing with HgCl_2 .² The solubility of HgCl_2 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.

²The Merck index lists HgCl_2 as a “violent poison” for which 1 or 2 g is frequently fatal. After using HgCl_2 (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.

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

1. Saturated aqueous HgCl_2 solution
2. 100 μl Eppendorf pipette with yellow tips
3. Plastic pipette with bulb, a cooking baster is ideal.
4. Swabbing tool (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

3. Sampling at Sea

3.1 Bottle Prep Procedure

The integrity of $\Delta^{14}\text{C}$ 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.

3.2 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 is possible should be written on the bottle label. This information may also be entered onto a data sheet; an example is shown in Figure 2.

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 WOCE water sampler, and any comments regarding unusual conditions.

3.3 Sample Transfer Procedure

The procedures described here are based on those used in C. David Keeling's laboratory for the collection of ΣCO_2 samples (Peter Guenther, pers. comm.) 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 remove the tope or laboratory wipe (always make sure the strips of tape have been removed before collecting any seawater). Place the tygon tubing on the Niskin bottle and flush with 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. Fill the bottle 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. Using this procedure, an AMS water sample will require approximately 800 ml of water.

When all the bottles have been filled, remove them to a safe, dry place and continue preparing the samples for storage in the following manner. Remove the stopper; wipe clean and dry, put 4 evenly-spaced strips of grease that extend approximately two-thirds of the way from the top to the bottom of the stopper; set the stopper aside. It is recommended that at least three types of Apiezon greases (L, M, and N) be brought along for use. The temperature at which one is sampling will dictate which grease to use. Although all these greases have the same working range (10–30°C), experience has shown that “L” is best at lower temperatures and “N” is best at higher temperatures. Using the large pipette or just by pouring, remove enough water for a 5 ml headspace to exist in the bottle; this level can be marked on the bottle. Using the Eppendorf pipette, add 100 μl of the saturated HgCl_2 solution to the bottle. Wipe the inside of the ground glass joint dry using the swabbing tool and place the stopper in the bottle. An alternate method of drying the joint is to use a laboratory wipe with your gloved hand. Care must be taken not to put your finger in the sample. Twist the stopper around while applying pressure to ensure that a good seal is made. Secure the bottle top with the red clip and with the rubber band. If a duplicate sample is to be taken, start filling the second bottle immediately using the same procedure. After both bottles are filled, shake gently to mix poison in.

3.4 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 the database. Useful information to include are: station number, latitude, longitude, sampling date and time, depth to bottom, sample bottle type (*e.g.*, rosette), cast number, rosette 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 securely, the plastic bag resealed, and the crate should be stored in a temperature-controlled environment. Seawater samples must not be exposed to extremes of temperature. **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 maintain their integrity, samples must be stored in a van which is capable of maintaining the temperature within this range. The samples should not be exposed to extreme temperatures during shipment either.

4. Laboratory Preparation of Samples

In this section, we discuss the methods used in our laboratory to convert the inorganic carbon in seawater to the graphite 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.

4.1 Laboratory Extraction of CO_2

Graphite targets for the accelerator are prepared from seawater in 2 steps; first, CO_2 is stripped from acidified seawater and, second, CO_2 is reduced to filamentous graphite. In this section, we will summarize the procedure we use to extract CO_2 from seawater; detailed procedures for each of the steps are on file at the AMS facility. The vacuum line used is shown in Figure 3.

4.1.1 Sample handling prior to stripping

Samples shipped to the AMS Facility are logged into our database upon arrival. We assume that any packing crate that has been shipped here may be contaminated with ^{14}C . The 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 the data sheet shown in Figure 4 is activated. The integrity of the greased seal on each bottle is checked as the sample is logged in.

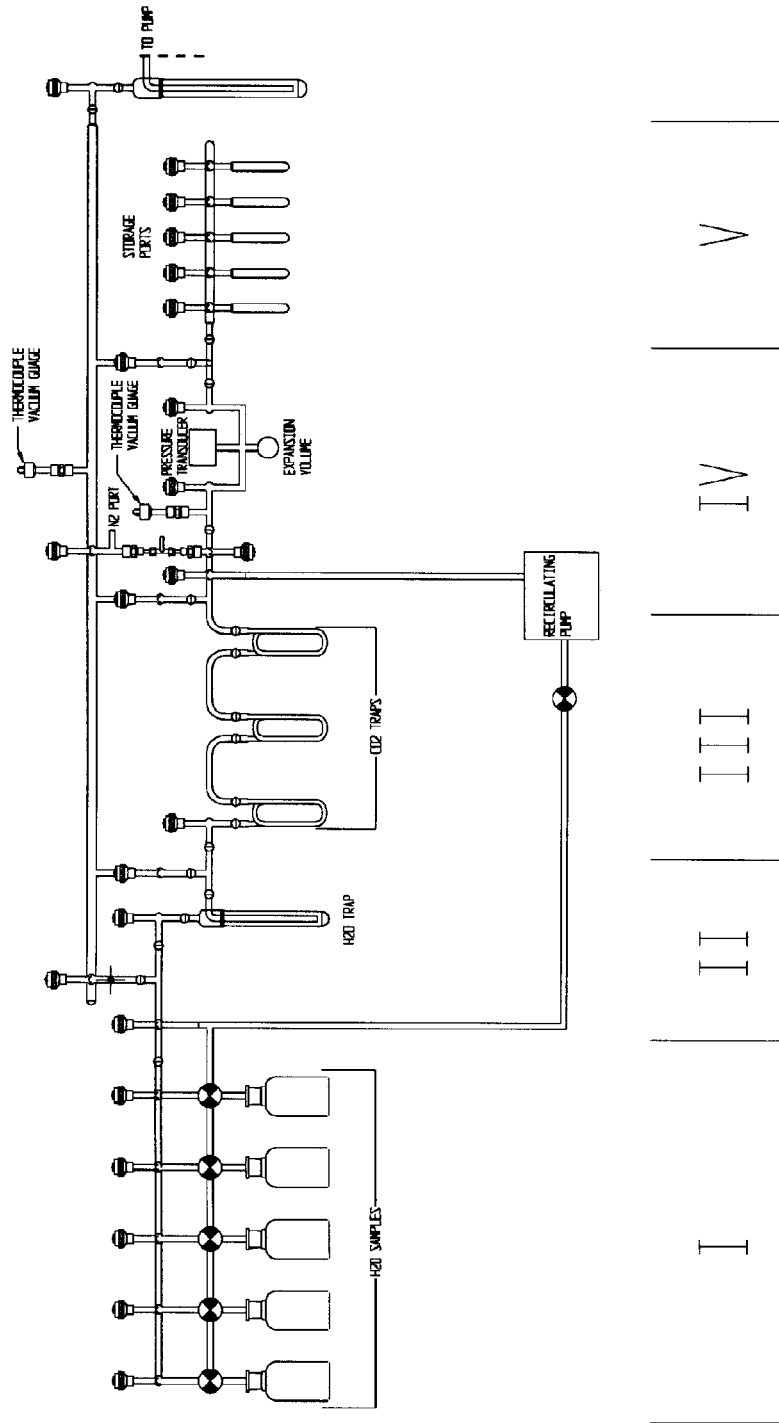


Figure 3: Water stripping line

AMS CHEMISTRY PREPARATION LABORATORY

SEAWATER ΣCO_2 ANALYSIS

Line #	_____	WS - #####	_____
Receipt #	_____	Method #	_____
Date:	_____	User:	_____
		R/D #:	_____
Sample ID.:	_____		
Sample Type:	_____	Seal Condition:	_____

SAMPLE STRIPPING

Port: _____

Start: _____

Total Stripping Time: _____

Stripping Rate: _____

CO₂ TRANSFER

Baratron Zero: _____

Sample: _____

Temperature: _____

Volume Expanded: _____

Storage Port: _____

ΣCO_2 Concentration

Wt. Bottle Full: _____

Wt. Bottle Empty: _____

Wt. Sample: _____

mmol CO_2 : _____

ΣCO_2 (mmol/kg): _____

COMMENTS: _____

SAMPLE SPLIT

Date: _____

Baratron Zero: _____

Sample: _____

Temperature: _____

^{13}C : _____

^{14}C : _____

mmol CO_2 : _____

COMMENTS: _____

Figure 4: Data sheet for water stripping

4.1.2 Preparation of the extraction line

Before working with any samples, the extraction lines (Figure 3) 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 N₂-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, regions I to III and the recirculating pump region are filled with clean CO₂-free N₂ gas to a pressure of approximately 0.8 atm.

4.1.3 Stripping the sample

At this point, the stripping procedure is ready to begin. The cold trap for water (Region II) and the cold traps for CO₂ (Region III) should be at the proper temperatures, -80° C and -190° C, respectively. When all the valves needed to recirculate the sample are open, 4 ml of 85% H₃PO₄ are added to the sample, the recirculating pump is turned on, and the sample is stripped for 10 minutes, this routinely produces a >95% yield of CO₂ from the sample. Water is frozen down in Region II and CO₂ is frozen down in the three traps in Region III. After stripping is complete, the inert gas is pumped away, the extracted CO₂ is purified by removing H₂O and uncondensable gases and transferred to Region IV where it is quantified in a known volume using a pressure transducer. The sample is then transferred to a labelled 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.

4.1.4 Sample 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 labelled with a bar code. One of the splits is transferred to the target preparation line, one is transferred to the VG PRISM stable isotope ratio mass spectrometer, and one is archived in a flame-sealed tube.

4.2 Preparation of Graphite Targets for AMS

Gaseous CO₂ is converted to filamentous graphite on the line shown in Figure 5; this line has a gas transfer region and a graphite reactor region. When the sample is transferred to the graphite preparation line, the data sheet shown in Figure 6 is activated. 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

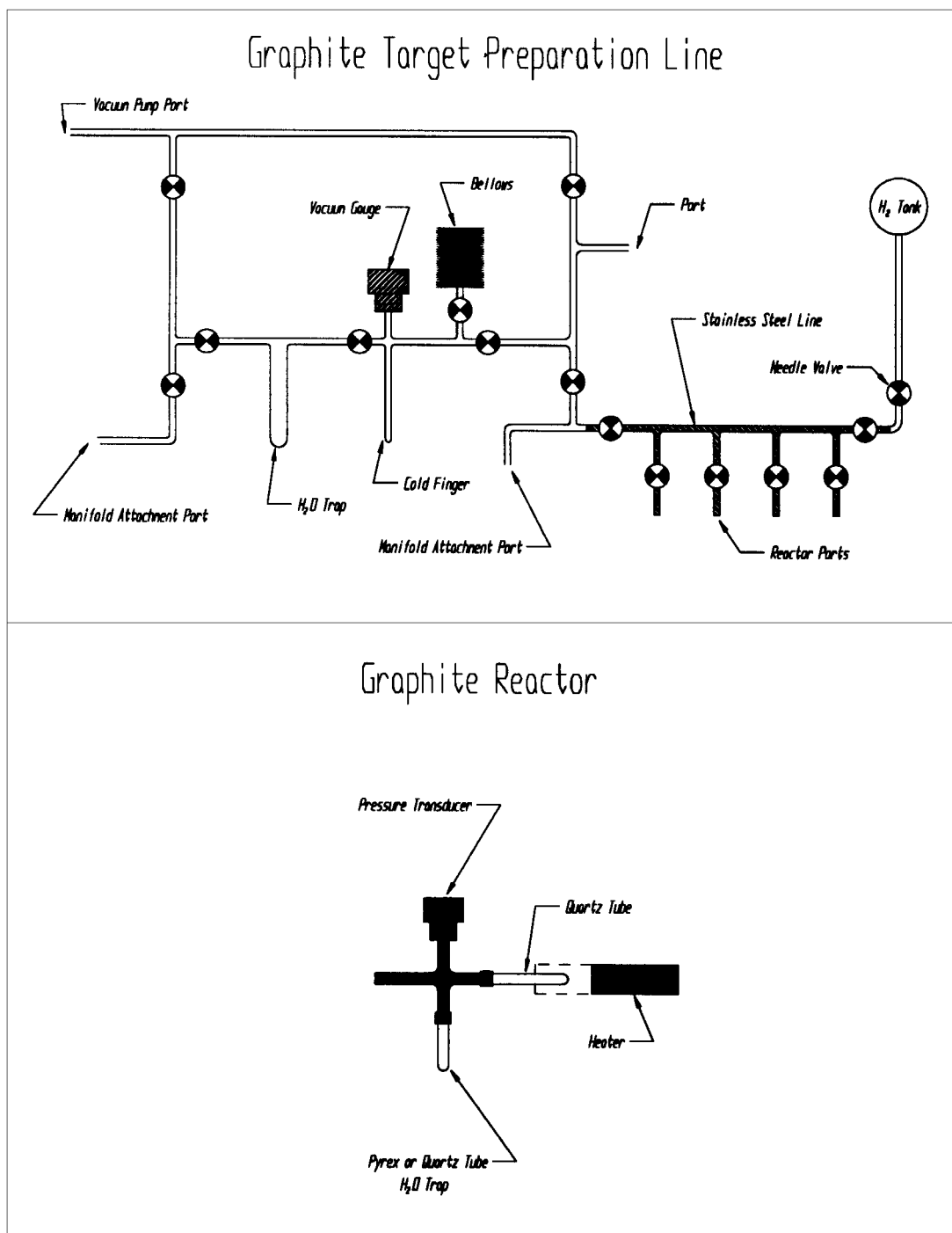


Figure 5: Graphite target preparation line

**NATIONAL OCEAN SCIENCES
Graphite Preparation Data Form**

OSG-####: _____ Date: _____
 RECEIPT #: _____ Operator: _____
 Sample ID: _____
 Method #: _____
 Reactor #: _____ Rxn Temp (°C): _____
 Reactor Volume (ml): _____ Start Time: _____

Catalyst: _____
 Wt. tube (g): _____
 Wt. catalyst (mg): _____

Sample BP (mmHg): _____
 Volume (ml): _____
 Temp (°C): _____
Total CO₂ (μmol): _____

H₂ Pressure (to add): _____
 Quality Graphite _____ Vessel Zero (atm): _____
 R/D #: _____ Initial P w/slush (atm): _____
 Final P w/slush (atm): _____

COMMENTS: _____

Figure 6: Data sheet for graphite target preparation

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. Because we are a new laboratory, we are working to improve all aspects of sample preparation and analysis. Thus, we are still optimizing our target preparation method to that most appropriate for the operating conditions of our accelerator. As such, we are not able to accept graphite targets prepared at other laboratories at this time.

4.3 Analysis of $\delta^{13}\text{C}$ of ΣCO_2

In addition to collecting CO_2 for the analysis of its radiocarbon content, splits of the extracted CO_2 can be analyzed for the stable carbon isotopic ($\delta^{13}\text{C}$) composition. This analysis is important for data calculations and will be useful in checking our methods with those of other laboratories. The AMS laboratory has a VG Prism stable isotope ratio mass spectrometer which is capable of analyzing $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ on a very small (< 0.5 μmol) samples of CO_2 to a precision of ± 0.02 and ± 0.06 ‰, respectively. The Prism is equipped with a 20 sample manifold with tube crackers for automated analysis of batches of samples submitted in valved vessels or flame-sealed tubes. We anticipate analyzing the $\delta^{13}\text{C}$ of only a statistically significant subset of the water samples we will process in the sample preparation laboratory.

5. Data Reduction and Reporting

Radiocarbon values of WOCE Samples will be reported as $\Delta^{14}\text{C}$ using established procedures modified for AMS applications (*e.g.* Donahue *et al.*, 1990). We expect the machine precision to be ± 3 ‰ from counting statistics and the overall precision of the complete analysis to be ± 5 ‰ or better.

6. Assurance of Data Quality

We are currently designing a comprehensive program to ensure the quality of the data from this laboratory. This program will include:

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

Some of the quality assurance samples will be standard seawater prepared in our laboratory that will be sent to sea and worked up as though it were a sample. This will allow us to check the “cleanliness” with respect to ^{14}C of the individual ships and sampling personnel.

7. References

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