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**Method Report for Total
Organic Carbon Measurements
GEOSECS**

By
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METHOD REPORT FOR

TOTAL ORGANIC CARBON MEASUREMENTS

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CONTENTS

ABSTRACT

The analytical method employed for the Total Organic Carbon (TOC) project in the Geochemical Ocean Sections Study (GEOSECS) is described in detail. The method generally follows that of Menzel and Vaccaro as modified by several subsequent workers including the GEOSECS group at Oregon State University which carried out the TOC project. Samples were drawn from 17 stations in the Atlantic and 35 stations in the Pacific. The cleanest shipboard conditions possible were maintained. The samples and reagents were sealed at sea into glass ampoules after purging the inorganic carbon dioxide. Completion of the oxidation, measurement, and data reduction was done ashore. The data have been transmitted via the GEOSECS Operations Group to the National Oceanographic Data Center. The precisions attained, including sampling errors, were 1.4 and 1.7 micro moles of organic carbon per kg (μM/kg) in the Atlantic and Pacific cruises, respectively (expressed as average standard deviations). Accuracy is estimated at plus or minus 10%. The mean TOC observed for the deep (>1 km) oceans was 35.3 μM/kg.

INTRODUCTION

This report details the analytical procedures employed for Total Organic Carbon (TOC) measurements on the Geochemical Ocean Sections Study (GEOSECS) program. TOC was measured for three purposes, 1) to complete the budget in the Sections of all carbon, both inorganic and organic, 2) to establish a baseline for the Sections against which to assess future addition of organic pollutants, and 3) to substantiate or negate at a higher level of precision, the findings of Menzel and Ryther (1968) that the concentrations of organic carbon, both dissolved and particulate, appear to be constant in the deep waters of the Atlantic and Pacific oceans.

Seventeen stations in the Atlantic and 35 in the Pacific were sampled for TOC. The number of water samples taken were 672 and 1,329, respectively. These were processed at sea for completion of the measurements in the shore laboratory at Oregon State University (OSU) according to the procedures detailed below. The data have been tabulated and sent to GEOSECS Operations Group for transmittal to the National Oceanographic Data Center. This Report summarizes parts of and augments the preliminary instruction manuals of Seifert et al. (1972, 1973).

GENERAL OUTLINE OF THE METHOD PROCEDURES

The method follows the one adapted for use in seawater analysis by Menzel and Vaccaro (1964). It has been further described by Strickland and Parsons (1968). Further modifications have been made by Fredericks and Sackett (1970), Schemel (1971), and in turn by our laboratory.

Broadly, the method is as follows. To 5 ml of sample in a 10-ml glass ampoule were added phosphoric acid and potassium persulfate. Inorganic carbon was removed as CO_2 by passing purified O_2 gas through the sample. The ampoule was sealed and organic carbon was oxidized to carbon dioxide by autoclaving the sealed ampoule. This carbon dioxide was stripped with nitrogen carrier gas and measured in a non-dispersive infrared gas analyzer.

Precruise Preparation of Materials

Cleaning of Equipment

All glassware, aluminum foil, reagent bottles, micro capillary pipettes, etc. were carefully cleaned and ignited in a muffle furnace for 4 hours at 475°C.

Prior to ignition, the ampoules were rinsed with distilled water and shaken nearly dry before the open ends were covered with aluminum foil squares. When the ampoules had cooled they were placed in packing cases.

An alternate cleaning method, used for equipment such as syringes, which could not withstand ignition, was treatment with chromic acid

cleaning solution at 70°C, rinsing with distilled water, and finally drying quickly in an oven or muffle furnace at low temperature.

Reagent Preparation

1. Organic-free water: Good quality distilled (not de-ionized) water, was redistilled in an all-glass[1] apparatus. This water (in 1.6 liter batches) was first refluxed with 13 g potassium persulfate and 3 ml 85% phosphoric acid for 4 hours. Then it was distilled into a freshly fired glass-stoppered bottle[1] discarding the first 100 and last 200 ml.

2. Phosphoric acid, 3% v/v: 30 ml analytical reagent grade 85% phosphoric acid was diluted to 1000 ml with organic-free water in a glass stoppered glass bottle.[1,2] After adding 10 g of potassium persulfate, $K_2S_2O_8$, the acid was placed (with stopper loosened) for 4 hours in a boiling water bath. After cooling, the acid was dispensed into pre-fired 20 ml glass ampoules and sealed. Once sealed, the acid ampoules were assumed to be stable indefinitely. One 20 ml ampoule contained enough H_3PO_4 to prepare 65 sample ampoules. However, in actual shipboard use, about 50% was lost to rinsing and "leftovers" at the end of each run. Fresh acid ampoules were always used for each cast's samples.

3. Potassium persulfate: Reagent grade potassium persulfate was sealed into 10-ml prefired ampoules for shipboard use. One persulfate ampoule

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[1] No grease was used on any of the ground joints of the apparatus.
[2] Add the acid to most of the water, mix, and then bring up to volume.

contained enough to prepare about 65 sample ampoules, but there was usually about 25% loss due to "leftovers." Commercially available "low-nitrogen" grade reagent was found to contribute higher carbon blanks than the usual reagent grade.

Shipboard Procedure

Cleanliness was the keyword. Great pains were taken to ensure a clean laboratory and "micro environment" for the TOC work. In the Atlantic a van was shared with only the water-library group and unused Nansen bottle storage. In the Pacific a portion of the wet hydrographic lab was partitioned off. Then, leg by leg of the cruises the following procedures were employed.

Preliminary Cleaning

At the beginning of each leg all exposed surfaces in the TOC lab were cleaned. Incoming air vents were wiped. The electrostatic air cleaner's filter grid was rinsed and dried.

The 30 liter Niskin water-sampling bottles to be used were rinsed with isopropanol, and the O-rings wiped prior to the first station of each leg. Before each cast, the spigots were rinsed with alcohol and wiped out with Kimwipes.®

No more than 12 hours, and when possible immediately before, each cast the 60-ml Pyrex glass sample bottles and their inverted standard taper ground joint caps were rinsed with distilled water and fired at 475°C for four

hours. The ampoules, cones, and purge tubes were also refired for four hours.

The syringes or Repipets were treated with chromic acid cleaning solution at 70°C, rinsed with distilled water, and then with the liquid to be dispensed (fresh seawater, deep sample or H_3PO_4). Stainless steel dippers used to measure out the persulfate and forceps used to handle purge tubes and cones were either fired at 475°C or cleaned with chromic acid, rinsed with distilled water, and dried. 6 $\underline{N}$ HCl was used to remove oxides as necessary.

New reagent ampoules were readied for use just prior to each cast coming over the side.

Sample Collection

The TOC samples were drawn from the Niskin bottles after dissolved gases and just prior to the helium samples. Seawater was allowed to flow through the larger of the two sampling spigots on the Niskin bottles for 15 to 20 seconds before the sample was drawn. The 60-ml sample bottles described and pretreated as above were used. The sample bottle was filled to just below the ground-glass joint with care to avoid contamination by contact of the hands with the bottle mouth or spigot. One sample bottle, similar to the others but of 125-ml volume, was filled from a previously sampled "deep" Niskin bottle to be used for preparing reagent blanks.

The ampoules were sealed as soon as possible in the same order as they were sampled. If stored for more than one or two hours, the samples

were refrigerated. After refrigeration they were allowed to warm up somewhat before pipetting to reduce volumetric errors.

Sample Preparation

Three glass ampoules for each sample were labeled using serially prenumbered labels. The station, time, date, cast, ampoule and Niskin bottle numbers, depths of collection and reagent batch numbers were noted on a data sheet. Potassium persulfate, 0.2 g, was added to the ampoules with a calibrated scoop followed by 0.25 ml of 3% phosphoric acid from a calibrated 1.0 ml Repipet[1] (Figure 1a). Five ml of the sample were then pipetted into the ampoules. Pipetting of the samples into the ampoules from the glass stoppered sample bottles differed for the Atlantic and Pacific cruises. On the Atlantic cruise a 10-ml Hamilton syringe with Teflon plunger and a Chaney adaptor set at 5 ml was employed. Ten cm long stainless steel cannulas cleaned with chromic acid were used with the syringes. On the Pacific cruise 10-ml glass Repipets were used (Figure 1b). Only Pyrex[2] glass, Teflon[3], and ceramic came in contact with the samples. The Repipets were blown to a standard taper joint (no grease!) to fit onto the sample bottles, obviating further transfer of the samples. The dead volume of the Repipet was reduced to facilitate flushing with new sample.

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[1]Registered trademark of Labindustries.

[2] Corning Glass Works.
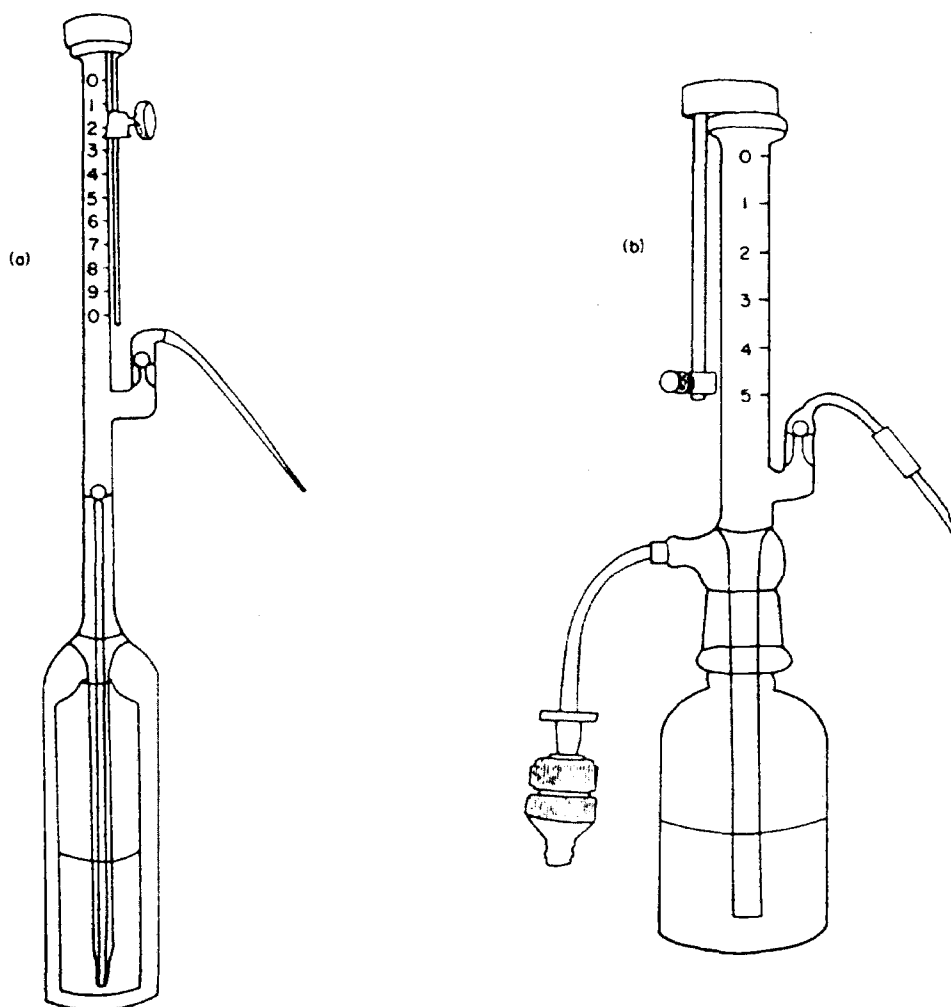
[3]A registered DuPont trademark.

Figure 1. Repipets used to dispense solutions and samples. (a.) The 1 ml
Repipet used for phosphoric acid reagent. An inner, capillary tube blown
to the Repipet dips to the bottom of an opened phosphoric acid ampoule. An outer
glass canopy ring-sealed to the inner capillary tube shrouds the ampoule and
protects it from dust. (b.) The 10 ml Repipet used for sampling seawater. It is
shown here fitted to a 60 ml sample bottle, with a standard taper joint
(no grease!). For use with blank preparations a second Repipet is used with a 125
ml boiling flask blown with a short neck to a mating standard taper joint. In both
cases, air is admitted to the flask or bottle through a small Millipore filter affixed to
the vent tube. This prevents the entry of dust. When not fitted with the Repipet the
sample bottles are covered with inverted standard taper caps.

Volumes of the syringes and pipets were checked throughout the cruises as detailed below, in the section "Volume Calibrations."

The operations to this point were carried out in an acrylic plastic dust box. Next the ampoules were transferred to an Oceanography International Corp. (1971) Purging and Sealing Unit (slightly modified at OSU, Figure 2). Each ampoule was purged of inorganic CO_2 for 5 to 7 minutes with O_2 before it was sealed. The time interval between reagent addition and ampoule sealing was kept as constant as possible. This time interval is important because the oxidation of labile organic compounds may begin, although slowly, from the time reagents and samples are mixed. Yet the inorganic CO_2 must be completely purged from the sample. Generally, while one set of ampoules was purging, the next set was being prepared.

The purge flow rate was approximately 70 ml/min. The oxygen was purified by passing it through a cupric oxide filled tube at 400°C. An autotransformer was used to control the temperature of the tube. After the initial adjustments, the flow of each purging tube was checked every month with a soap film-burette flow meter. If the flow rate was less than 70 ml/min, purge gas pressure was increased to restore the proper rate. Sealing of the ampoules was done with a 4-tip oxygen-propane microburner. A sooty flame was avoided at all times to prevent contamination of the laboratory atmosphere. The upper neck of the ampoule was held by a clamp and the lower portion of the ampoule drawn away from the neck after the
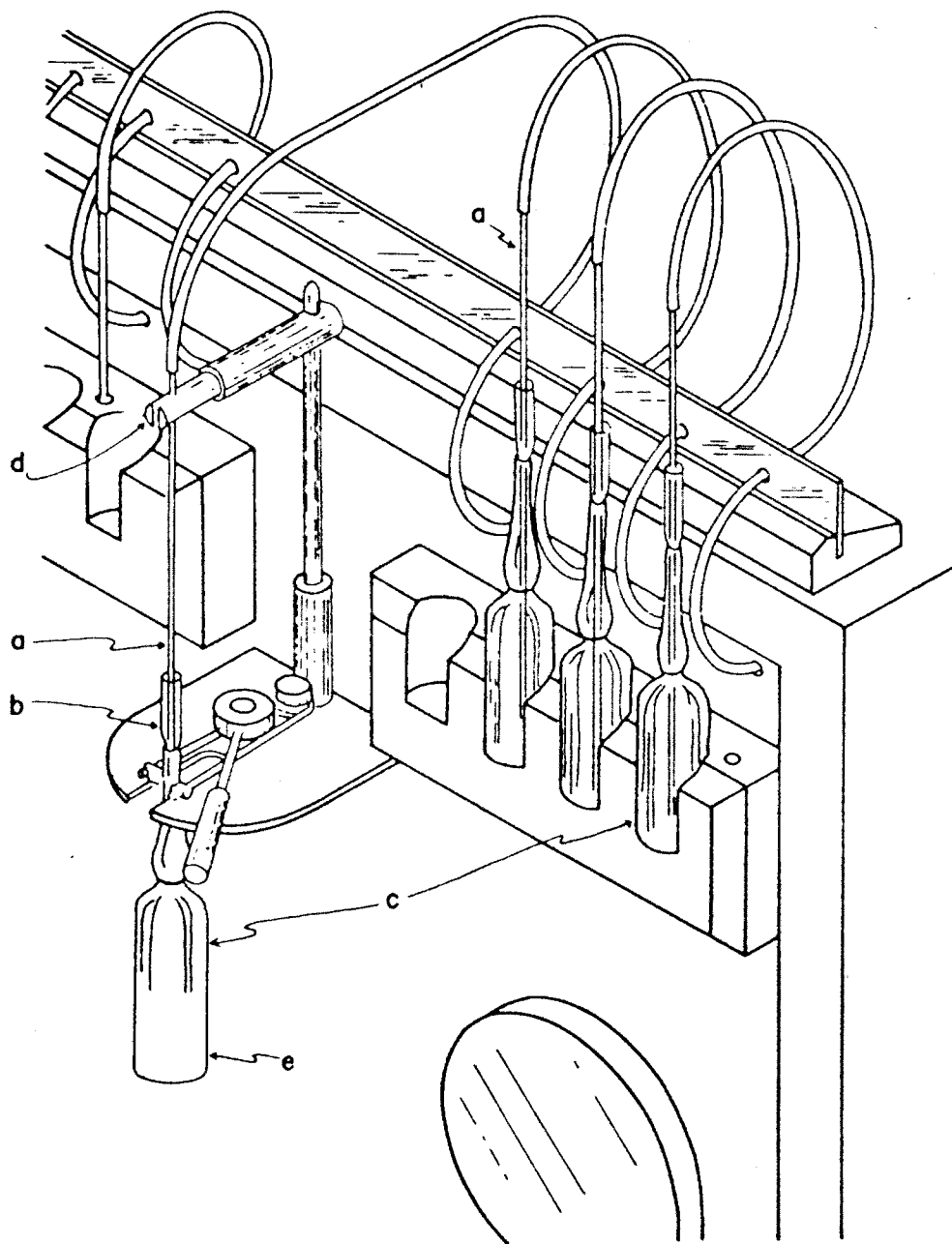
8

Figure 2. Detail of part of the Purging and Sealing Unit. Several purge tubes (a) are shown inserted through purge cones (b) into ampoules (c). The purge tube clamp is shown at (d). Redrawn from Oceanography International Corporation (1971).

glass became molten. "Purge cones" were used to minimize contamination during the purging and sealing operations.

Blank Preparation

The blank is that portion of the carbon dioxide arising from organic matter in the reagents, and on the ampoule and bottle walls, and sampling apparatus; it must be subtracted from the gross amount of carbon dioxide measured. The magnitude of this blank was usually between 5 to 8 μM/kg. Ampoule sets for blank estimation were prepared during most stations in the Atlantic, and every station in the Pacific using seawater from any sample deeper than 1000 m and more than 300 m from the bottom. The cast and depth of this water were noted on the data sheet.

Volumes of seawater for the blank determinations were measured differently on the Atlantic and Pacific cruises, analogously to the sample volumes. In the Atlantic a 10-ml Hamilton syringe, like that for the samples but set at 2.5 ml, was used. It was clamped to the inside of the sampling dust box, and was fitted with a three-way Hamilton Miniature Inert Valve in which only Teflon and polychlorotrifluoroethylene plastic contact the samples. A chromic acid cleaned Teflon tube dipped into the sample bottle to draw sample water which was dispensed into the ampoules through a 10-cm stainless steel cannula. For the Pacific cruise a Repipet similar to the sampling Repipet was employed. It was adjusted to 2.5 ml. In order to provide sufficient water for flushing and the several blank preparations, a 125-ml reservoir was used instead of the usual 60-ml sample bottle. After

three preliminary rinsings of the syringe or ten flushings of the 2.5-ml Repipet, three or four ampoules each were prepared, with reagents and 2.5, 5, and 7.5 ml water volumes. These were then purged and sealed as were the samples. As the ampoules were sealed, their numbers were checked and sealing times noted on the data sheets.

Volume Calibration

Once during each station, duplicate ampoules were prepared with 2.5 and 5.0 ml each of seawater sample and the water temperature and sample number recorded. The delivery volumes were measured by weighing in the shore lab. Volumetric errors were corrected when the final carbon concentrations were calculated.

Transporting Samples to Shore Laboratory

After the ampoules were sealed they were returned to the packing cases along with the original copies of the data sheets. Any poorly sealed ampoules had tell-tale salt deposits at the sealed tips upon arrival in the shore lab. No special storage or shipping precautions were necessary beyond care to avoid breakage.

Shore Laboratory Procedure

The shore laboratory task was to make the carbon dioxide measurements on the ampoules from the cruise, intermixed with standards prepared ashore. Blank, standard, and sample data were then combined as detailed below and the data reduced. Quality control was exercised taking into account notes made by the analysts at sea and ashore to remove clearly unacceptable data. A description of the instrumentation and procedures follows. Figure 3 shows a schematic diagram of the instrumentation.
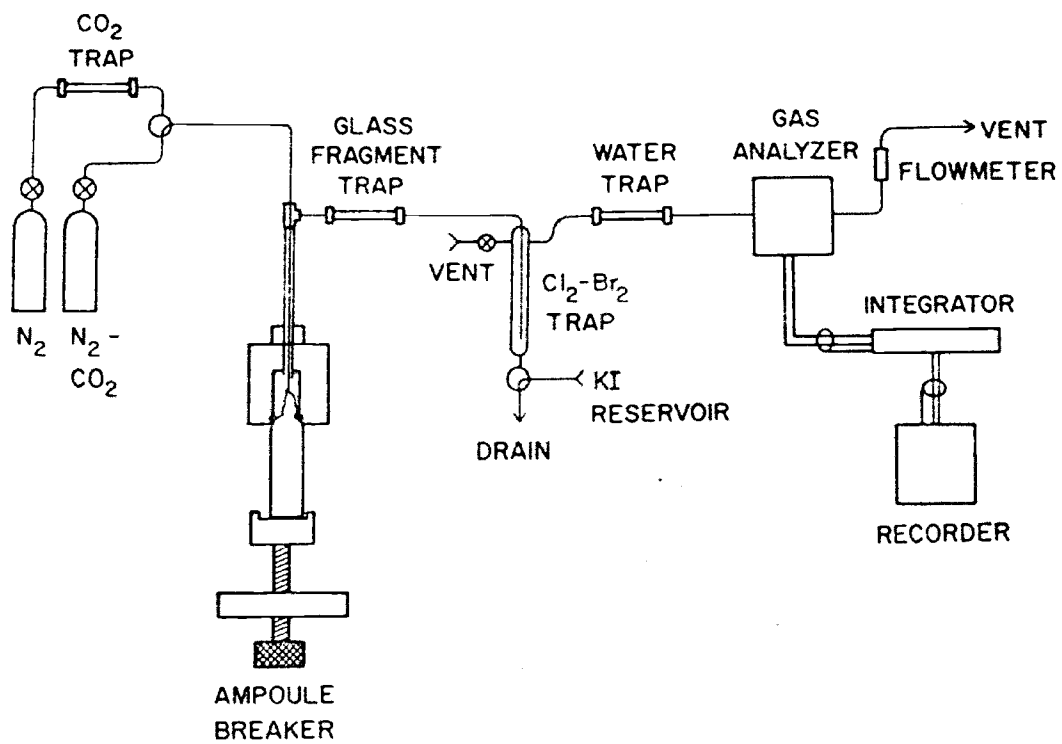
Figure 3. Schematic of the shore-based analysis unit.

Analyzer

A Mine Safety Appliance LIRA Model 200 or 300 non-dispersive infrared gas analyzer (IRA) was used to analyze the ampoule contents for carbon dioxide produced by the oxidation of organic matter. The IRA was used with a Hewlett-Packard 3373B electronic integrator and a 1-millivolt Honeywell strip chart recorder.

Ampoule Analyzing Module

This unit contained pre-purified nitrogen gas and span gas controls and conditioners, an ampoule breaker assembly, and gas flow meter. Activated charcoal, molecular seive and Ascarite removed CO_2 and volatile organic impurities from the nitrogen carrier gas. A glass wool filter, after the ampoule breaker, removed glass fragments then a glass trap filled with an acidic aqueous potassium iodide (KI) solution removed chlorine and bromine. Lastly Anhydrone drying tubes were used to remove water vapor from the nitrogen stream before it entered the analyzer.

The KI trap removed the chlorine and bromine formed from sea salts during the oxidation of the organic matter. This protects the infrared analyzer cells from corrosion by the halogen gases. The trap contained a solution of 20 gm KI per 50 ml of 10% H_2SO_4.

Autoclaving

When unpacked, the ampoules were inspected for evidence of leakage. The ampoules were then autoclaved at 130°C (25 psi pressure over liquid water) for 4 hours in an electric autoclave to oxidize the organic matter to

13

CO_2. A constant but rapid heating rate and slow cooling rate were necessary to avoid ampoule breakage.

Measurement Procedure

1. The LIRA 200 or 300 was turned on at least 24 hours before the measurements were begun. The recorder and integrator needed only a few minutes warm-up.

2. The loosely packed Anhydrone drying columns were replaced as needed to maintain an unrestricted flow of dry gas into the analyzer.

3. Flow rates of the stripping and span gases were adjusted to 200 ml/min flow rate through the system, and regularly checked and readjusted as necessary.

4. The LIRA 300 was aligned to zero with the stripping gas (CO_2-free N_2) and full (96%) scale using 400 ppm CO_2 in N_2. This alignment allowed analysis of concentrations of up to about 170 μM/kg. For more carbonrich samples, the instrument sensitivity was reduced to permit the measurement of about 340 μM/kg, if necessary, without realignment.

5. After placing a new ampoule in the chamber and sealing it against a neoprene "O" ring the system was purged with the stripping gas until a zero recorder trace was obtained.

6. After the ampoule neck was crushed by a 1/4" OD x .035" wall stainless steel tube, an 1/8"-tube carrying the stripping gas was then inserted to the bottom of the ampoule.

7. The gas was stripped of Cl_2 and Br_2 and moisture when it passed

14

through the KI trap and Anhydrone column respectively, minimizing corrosion and the need for cleaning of the IRA sample cell.

8.   The signal produced by the CO_2 passing through the LIRA was visualized as a peak on the strip chart recorder and the peak area was measured by the integrator.

9.   At least three standards of different concentrations were analyzed at the beginning of each day's analyses to establish that the instrument was working properly; subsequently, a standard was analyzed after every 8 to 10 samples.

10.   Samples, standards, and blanks in quasi-random order were all analyzed in this manner.

Standard Preparation

A 1.0 gC/l stock solution was made by dissolving 0.250 g of dry glucose, (dried under vacuum at 70°C overnight), in 100 ml of organic carbon-free water, then adding mercuric chloride, $HgCl_2$, and mixing before refrigeration. This stock solution was pipetted without further dilution with ignited micro-capillary pipets (μ-caps).

Integrity of calibration after ignition was verified on several batches of μ-caps by weighing distilled water contained. After the μ-cap was picked up with clean forceps, one end of it was touched twice to the surface of the standard solution and allowed to fill by capillary action. It was dropped into an ampoule containing 5 ml of organic carbon-free water, 0.25 ml 3% H_3PO_4, and 0.2 g of $K_2S_2O_8$ without touching the sides of the ampoule neck.

The ampoule was purged and sealed in the same manner as the samples.

Using the 1 gC/l glucose solution, standards of the following concentrations were prepared and measured intermixed with the samples:

1 lambda μ-cap gave 0.2 mg /l added

2 lambda μ-cap gave 0.4 mg /l added

3 lambda μ-cap gave 0.6 mg/l added

4 lambda μ-cap gave 0.8 mg /l added

5 lambda μ-cap gave 1.0 mg /l added

A "zero" standard solution consisted of 5 ml of the diluent water for these standards.

Calculations

The calculations to obtain standard curve slopes, blanks, sample concentrations in μM/kg, and precisions are outlined below. They were performed on a WANG 600 programmable desk calculator. The blank, B, to be subtracted from the integrator counts, S, for various samples was obtained by regressing the counts produced by the incremental volume blank ampoules on the volumes taken for those ampoules.

16
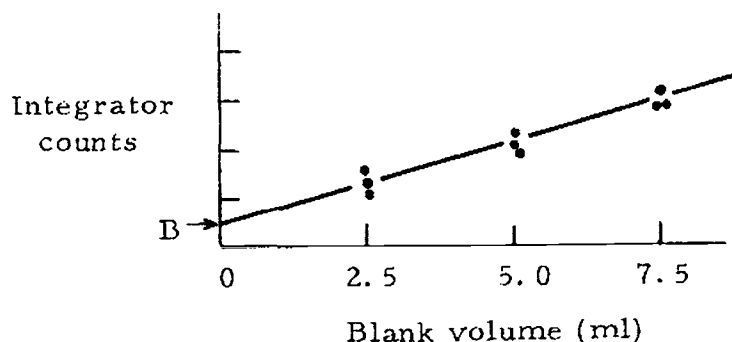
For the standard curves counts for the standards were regressed on the added standard concentrations to obtain the reciprocal slope, F, which was used to calculate concentrations.

Added standard concentration (mg C/l)
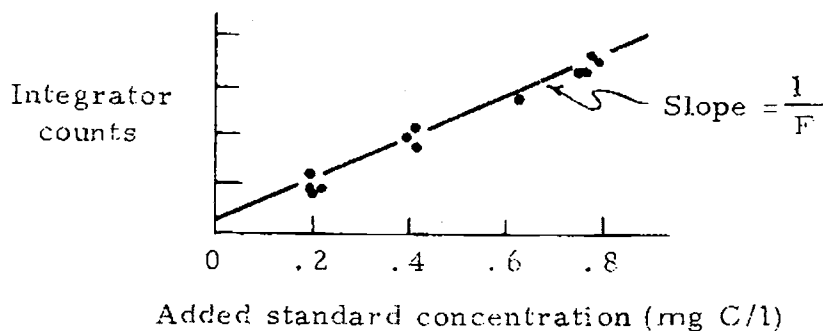
To calculate sample concentrations:

C = (S-B)(Counts) x F (mg C/l/count).

The mean of each group of replicates and the precision of that group were also calculated. Finally, from the atomic weight of carbon and the density for each seawater sample at 20°C and its salinity, the concentration in mg C/l was converted to μM/kg.

ACCURACY AND PRECISION

Precisions have been estimated in several ways for both the Atlantic and Pacific cruises.

The precision of a single measurement of a single sample was derived from the replicate ampoules sealed for each water sample. Grand averages of these estimates for the Atlantic and Pacific cruises (for samples from deeper than one km only) are 2.4 and 2.2 μM/kg (1 S.D.) respectively. The standard errors of the means of the usual triplicate measurements made on the samples thus become 1.4 and 1.3 μM/kg, respectively. Occasionally, as noted in the data lists samples were measured singly, in duplicate, or quadruplicate and the standard errors vary accordingly for these samples. Using the mean deep water TOC observed on the GEOSECS cruises (35.3 μM/kg), we find relative standard errors for both the Atlantic and Pacific of 4%, for the means of the triplicate analyses.

Drawing of several samples from the same Niskin bottle did not add significantly to the errors.

The sampling errors introduced by use of the GEOSECS Niskin rosettes was estimated by assuming that all of the water column at a given station had a uniform TOC concentration below 1 km. A standard deviation from the mean was then computed for all the deep samples at each station. Finally, a grand mean of all station standard deviations was computed for each ocean. The result was as follows: Atlantic, 1.4 μM/kg; Pacific, 1.72 μM/kg. Apparently in the Atlantic the GEOSECS sampling did not introduce

a significant amount of scatter. However on the Pacific cruise the errors introduced in sampling are statistically significant.

The accuracy of organic carbon measurements in seawaters is a matter of controversy. Basically, there are two methods of measurement and each has its school of proponents. Both methods involve freeing the sample of inorganic carbon (CO_2, HCO_3^-, and CO_3^{-2}), combusting the organic carbon to CO_2, and finally measuring the amount of CO_2 produced. The two methods differ in the mode of combustion, one involving a high temperature (400°C or more) "dry" combustion in an oxidizing atmosphere (for instance see Sharp, 1973), the other a "wet" combustion in aqueous solution by strong oxidizing solutes at modest temperatures (100-130°C). The latter method is the one reported here.

Proponents of both wet and dry techniques acknowledge loss of volatile compounds by either technique. Most estimates of the maximum fraction of such compounds present in the total organic matter present in usual seawater samples are given as an upper limit of 10% or less. (See Swinnerton and Linnenbom, 1967; Corwin, 1970.)

Also common to both methods are sampling errors. These can be so large as to be several orders of magnitude larger than the natural levels of TOC measured. In the present work, notes were made in the field logs when sources of contamination such as surface oil slicks at the time of launching or recovering the samplers were observed. Samples below 200 m were considered to have been contaminated when as isolated single measurements they were higher than a smooth curve drawn through the adjacent points by five or more average standard errors of the mean of

two or three ampoules. Thus a single TOC value approximately 8 μM/kg higher than the smoothed profile was discarded as having been contaminated. A uniform low level contamination of the samples would not be detected by this procedure. At this writing we have no way of evaluating such contamination. This error is in the opposite direction to that from the loss of volatile constituents.

Sample contamination after sampling and during processing can be evaluated by our blank procedures and corrections have been made for this effect. Therefore the systematic error introduced by this source should be insignificant.

We estimate systematic analytical and sampling errors to be less than 3.5 μM/kg or 10 percent at the 35 μM/kg deep-sea TOC level.

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