THE DETERMINATION OF TOTAL ORGANIC CARBON IN SEAWATER

by

Gary N. Peterson

John R. Montgomery - Supervisor

Harbor Branch Foundation
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INTRODUCTION

One of the most important chemical processes known to man is assimilation. This basic component of life involves the creation of organic substances by living organisms from the chemical components of inorganic matter. In the sea, plankton is the main producer of new organic matter from inorganic compounds. The process is composed of two basic functions. The first involves the decomposition of plant and animal materials which may result in an initial 15 to 50 percent loss of total biomass (T.R. Parsons and H. Seki, 1968). The second function is the excretion of extracellular products by living plants and animals. The term excretion has come to include not only the true excrement of animals, but also the liberation of soluble organic materials by plants. In some species as much as 50 percent of the photosynthetically fixed carbon dioxide may be released as soluble organic carbon. The organic compounds contributed to the carbon reservoir, either through decompositon or excretion, could be quite appreciable.

Duursma (1963) proposed that the level of dissolved organic matter may be used as an index of primary productivity by measuring its rate of accumulation.

In coastal regions, the amount of organic carbon contributed as soluble and particulate material may be considerably higher due to the addition of terrigenous material washed from the surrounding land or from pollution by a domestic or industrial outfall.

Furthermore, organic carbon is an important chemical factor because of its influence on many inorganic substances. There is direct analytical evidence that many of the metal ions in seawater may be partially associated with organic matter. It has been shown that degradation of the organic matter in seawater results in an increase in the amount of free metals such as copper and mercury (Williams, 1969; Foster and Morris, 1971). In some cases, covalently bonded organometallic compounds will occur. These complexes may be produced microbiologically. More frequently however, the metals occur in co-ordination compounds with organic substances released from the cell. Slowey et al. (1967) have shown that 8.4 to 56 percent of the copper in seawater is extractable by chloroform. Barsdate (1970) found that more than half of the zinc, cobalt, and manganese in lake water was associated with large organic molecules incapable of passing through a dialysis membrane. The precipitation of calcium carbonate also appears to be regulated by the organic fraction. As the organic content of seawater is increased, the calcium carbonate precipitates become coated with a layer of organic material thus limiting their capacity to act as sites for further precipitation.

Most of the available information on organic material is in terms of organic carbon. The carbon measurement usually involves destruction of the organic matter by either wet or dry oxidation or by ultraviolet irradiation, followed by measurement of the carbon dioxide evolved. There have

been many technical difficulties in the development of the methods, and the results obtained by different techniques are still not precisely comparable. Methods do exist, such as gas chromatography and fluorometry, which display good sensitivity and precision for simple organic compounds or classes of compounds. However, the organic material in seawater is so diverse that no single detector is sufficient. This is the justification for conversion of all the organic material to one compound such as carbon dioxide, which is easily measured with a single detector.

The analysis techniques fall into two basic categories: dry combustion and wet oxidation. The dry combustion method has evolved from a technique devised by Skopintsev and Timofeyeva (1962). The sample is heated from 600° to 900°C in the presence of an oxidizer and the resulting carbon dioxide is measured. The technique requires that the water must be removed from the sample either prior to or during the analysis. The drying step is long and tedious. In 1967, Van Hall and Stenger improved the technique by developing a direct injection method. A sample is injected directly into a combustion furnace in a stream of oxygen. The resulting carbon dioxide is dried and measured by a nondispersive infrared analyzer. The method worked well for fresh water with a high organic carbon content and an instrument was soon available commercially. The main disadvantage of this method is that it was not suited for seawater. The lower limit of sensitivity was about at the upper limit of organic carbon

in seawater. The procedure also suffered from back pressure due to the injection of the water vapor and was limited by the small sample size. Later, Sharp (1973) made some major changes in the size and shape of the combustion tubes and in the method of retaining the sea salts. This improved the technique enough to be used for seawater with reasonable precision.

Another approach to dry combustion is the freeze-drying technique developed by Gordon and Sutcliffe (1973). In this method, the sample is acidified and taken to dryness in a lyophilizer. The need to freeze-dry large volume samples makes this a time-consuming method. The dry combustion technique still has some disadvantages, however. The infrared analyzer is required to operate at its sensitivity limit. Achieving precision of even ten percent requires a great deal of "tender loving care."

The more popular technique, wet oxidation, is not affected by salt in the sample. The basic procedure involves removing the inorganic carbonates from the sample, usually by acidification and purging with oxygen or nitrogen. A strong oxidant is added to the solution which is then heated or treated by some similar method to accomplish oxidation. The carbon dioxide generated from the organic carbon is then removed and measured. The main variations in the method have been in the oxidants used and the method of measuring the carbon dioxide.

Some of the oxidants used have been silver dichromate, potassium peroxide, dichromate in sulfuric acid, and hydrogen peroxide. Changes in oxidant were made to speed up the analysis and to achieve more complete oxidation.

Armstrong et al. (1966) developed a photochemical oxidation technique using high intensity ultraviolet light. However, the use of potassium persulfate as an oxidant as described by Menzel and Vacarro (1964) has become the standard method.

Early methods for the carbon dioxide determination included gravimetric and volumetric techniques. More recently, conductometric, spectrophotometric, chromatographic, and mass spectrographic methods have been used. Presently, the most commonly used instrument is the non-dispersive infrared gas analyzer which is extremely sensitive and selective.

Although the Menzel and Vacarro method has become the accepted technique, it too has its drawbacks. Sharp (1973) has suggested that the addition of persulfate and subsequent degassing may lead to a loss of some volatile organic compounds. Furthermore the wet oxidation technique seems to produce an overall lower result than the dry combustion method. This would suggest a lack of complete oxidation by the wet method. It has been argued that the wet oxidation method, though incomplete, measures the biologically useful dissolved organic carbon.

There is presently no flawless refereed method for the measurement of organic carbon in seawater. The best way for an analyst to interpret his data is to understand the chemistry of his chosen method and to realize the imperfections of the technique.

The Oceanography International Total Carbon System, using the Menzel and Vacarro wet oxidation method, was chosen by this laboratory. The main attributes of the system are its rapid sample analysis capability. The following report describes the Total Carbon System and its use.

MATERIALS AND METHODS

This technique is based on the wet oxidation method developed by Menzel and Vacarro (1964). The procedure involved removing the inorganic carbonates and carbon dioxide from the sample. This is accomplished by the addition of phosphoric acid and subsequent purging of the sample with oxygen gas. The organic carbon is converted to carbon dioxide by oxidation with potassium persulfate enhanced by heating the sample to 175 degrees centigrade. The resulting carbon dioxide is removed by purging the sample with nitrogen gas. The displaced carbon dioxide passes through a vapor trap to remove gross amounts of water vapor. Further drying is accomplished by passing the carrier gas stream through two columns of granular magnesium perchlorate. Finally, the carbon dioxide is measured as it is passed through a nondispersive infrared analyzer. Samples are prepared, digested, and analyzed in glass ampules.

INSTRUMENTATION

The instrumentation used for this carbon analysis procedure is the 0524B Total Carbon System designed and produced by the Oceanography International Corporation. The system consists of two main units. The first is the ampule purging and sealing unit. This unit purges the ampules of inorganic carbon compounds by bubbling the sample with purified oxygen. The oxygen is "scrubbed" of impurities

by passing through an ascarite column and an eight inch catalyst tube containing cupric oxide heated from 450 to 500 degrees centigrade by a 192 watt element. An oxygen-propane microburner is used to seal the ampules. A clamping assembly holds the top of the ampule and the lower portion is twisted and lowered as the microburner melts the neck of the ampule. Glass "purge cones" placed over the ampules prevent contamination from carbon dioxide during the sealing operation.

The other main component of the system is the ampule analyzing unit. This instrument consists of a Model 303 Lira nondispersive infrared analyzer. The infrared energy within this unit is generated by applying approximately 2.6 volts d.c. across two helices of nichrome wire. This energy is split into two parallel beams, each passing through a cell. After passing through either the sample cell or the reference cell, the energy strikes a detector which transforms the optical signal to an electrical signal. When the gas to be analyzed is carried into the sample cell, it absorbs some of the infrared energy at the preselected wavelength. This absorbance creates a difference between the two beams from which the detector produces a signal. The signal is amplified, rectified, and output directly to an integral meter.

The output current from the infrared analyzer goes directly to an electronic integrator which converts the potential to a frequency. A running sum of the cycles from

this frequency is shown on a five-digit LED display. The resulting number is directly proportional to the intensity and duration of the carbon dioxide measured by the analyzer. A simple peak height assessment would fail to provide any indication of the carbon dioxide residence time in the sample cell.

The carrier gas used in the analyzing unit is simply industrial grade nitrogen. An ascarite scrubber is used to remove any interfering carbon dioxide from the nitrogen. Drying tubes are used to remove water vapor from the carrier and carbon dioxide gasses. Granular magnesium perchlorate is used as a drying agent in the second and third tubes. The first tube is a vapor trap to remove gross amounts of water. This glass tube is not packed with the drying agent.

Finally, the analyzing unit is complemented by a Houston Instrument strip chart recorder. This instrument graphically displays the signal generated by the infrared analyzer. A Model 0512DP Electronic Printer is also supplied to print the integrated signal of each sample.

INSTRUMENT PREPARATION

The set up and instrument preparation of the Total Carbon System is fairly simple. The purging and sealing unit is made ready for operation by connecting the gas port to a cylinder of commercial oxygen. The pressure reducing regulator on the cylinder should be adjusted to deliver 20 p.s.i. The input voltage to the catalyst heater must be adjusted to approximately fifty volts to maintain 450 to 500

degrees centigrade. This may be accomplished by use of a Model E1010VA STACO variable power supply. After attaching the propane bottle to the valve on the back of the cabinet, the purging and sealing unit is ready for use.

Attach a cleaned, glass purge tube to each of the eight purge gas lines. By heating one end of the glass tube, it may be inserted into the Teflon^{R} purge line. The purge tube should be handled only by the end to be attached to the gas line to avoid contamination. Once attached, the purge tube may be passed through the large end of a glass purge cone. The end of the glass tube may be placed in one of the small holes in the wooden rack to retain the purge cone.

The ampule analyzing unit must also be connected to the appropriate gas supplies. Two gas ports are located on the lower right side of the cabinet. The port toward the front of the unit is the compressed air connection. The function of the compressed air is to calibrate or "span" the infrared analyzer. Therefore, the gas must contain a specified amount of carbon dioxide. The special ordered bottle of compressed air must be supplied with an assay showing a carbon dioxide content of 300 to 350 parts per million. The pressure regulator for the compressed air should be adjusted to 20 p.s.i. The other gas port is for commercial grade nitrogen. Set the pressure for this purge gas at 20 p.s.i. also. Polyethylene tubing is supplied with the unit for the gas connections.

Plug the power supply cord into a regular 110 volt outlet. A voltage regulator may be used to provide a constant power input. The infrared source requires a four to five hour warm-up period. It is, therefore, recommended that the power switch for the infrared analyzer remain in the "ON" position. The electronic integrator may be turned on and off as needed.

The ampule breaking assembly must be assembled before the instrument can be calibrated. The cutting plunger is the stainless steel piece with the four jaws on the end. Gum rubber o-rings are supplied to be seated in the grooves just above the jaws. A small amount of silicone grease may be applied to the o-rings. Drop the jawed end of the plunger through the opening in the plexiglass support attached to the top of the wooden block. A glass hood may be recognized by the flange at one end. Slide this piece, straight end first, over the jaws and o-rings of the cutter plunger. Insert a small plug of glass wool into a short piece of 3/16" I.D. Tygon tubing. Attach one end of the tube to the gas port on the side of the cutter plunger. The other end should be connected to the white, nylon gas tube protruding from the front of the cabinet. Connect a stainless steel purge tube to the clear gas line. Place one of the small, blue, rubber plugs in the fitting <u>under</u> the knurled nut on top of the plunger. Replace the knurled nut loosely. Insert the purge tube into the hole in the nut, through the hole in the rubber plug, and down into the plunger. A small

amount of silicone grease may be helpful. Tighten the nut, with fingers, until a good seal is achieved and the purge tube may still be moved up and down. The cutting assembly is completed for gas flow by placing either the standardization vial or an ampule in position.

To use a sealed ampule, place a gray plastic adaptor and then a gum rubber seal over the neck of the ampule. The ampule is put into position by inserting the neck inside the glass hood of the cutting assembly. Tighten the threaded elevator against the bottom of the vial until a good seal is achieved. The ampule may have to be turned slightly in order to seat the rubber seal to the glass hood.

The difficult part of the instrument preparation is packing the drying tubes. The tubes are located to the right of the cutter assembly. The first tube, on the left, is a vapor trap. Do not pack this one. Simply place the empty glass tube against the upper spring loaded connection. Push up and place the bottom of the tube over the o-rings at the base connection. Check both top and bottom for good seals. The other two tubes must be filled with granular magnesium perchlorate. Insert a small plug of glass wool to about one inch from one end of the glass tube. Pour a small amount of the drying agent into a convenient pouring vessel. A small plastic weigh boat works well for this purpose. Select the granular crystals and discard the powder. The very fine material tends to pack too tightly and restricts the gas flow. Fill the tube to within about an inch and a

half from the top with the crystals. Tap the glass lightly while filling to uniformly pack the column. Do not pack the drying agent too tightly. It will require some experimentation to achieve the proper gas flow through the packed tubes. Place another plug of glass wool at the top of the tube. Insert the tubes into the two remaining positions in the same manner as the vapor trap.

The Houston Signal Recorder should be connected to a 110 volt outlet. Attach the recorder leads to the appropriate red and black terminals on the analyzer unit just above the gas ports. Adjust the recorder signal to correspond to the meter output. This is done by adjusting resistor 91 as directed in the Lira manual on pages 2-12, step 7.

The final step toward preparing the analyzing unit for operation is interfacing the electronic printer. Connect the power cord to a 110 volt outlet. Attach the interface cable to the lower terminal on the back of the analyzer unit.

CALIBRATION OF INSTRUMENT

At this point, the instrument is ready to be zeroed and calibrated. Turn on both the nitrogen and compressed air bottles. Adjust the regulators to 20 p.s.i. Check all connections for leaks.

1. Pull the two-way valve out to the "IR ANALYZE" position.
2. Flip the zero gas and flow valves to the "ON" position (handle straight out). This allows nitrogen, the "zero gas," to flow through the system to the analyzer.
3. Adjust the flow rate with the IR rate valve to 200 ml/min or a reading of 13 on the rotometer.
4. Allow the system to purge for two to three minutes, then zero the meter on the analyzer. This is accomplished by loosening the locking wheel on the zero adjust knob and turning the knob until zero is reached.
5. Close the zero gas toggle valve.
6. Open the span gas toggle valve. This allows the compressed air or span gas to flow through the system.
7. Adjust the delivery pressure of the span gas if necessary to achieve a flow rate of 200 ml/min or 13 on the rotometer. Note that there is no rate control for the span gas on the instrument.
8. Allow the system to purge with span gas (compressed air). This should cause a deflection of the meter on the analyzer.
9. After the meter has settled to its maximum point, use the span control knob to set the proper calibration setting. This setting may be determined from the calibration curve supplied with the instrument. For example, span gas with a concentration of 300 ppm carbon dioxide should produce a meter reading of 67. The actual meter setting is not extremely important.

However, it must be set to exactly the same position each time the instrument is calibrated. One may choose to adjust the meter to 100, or full scale, as the calibration setting. However, this may lead to off-scale readings for the higher concentration range samples.

10. Close the span gas valve.
11. Open the zero gas valve and recheck the zero adjustment.
12. Recheck the span gas meter reading and then allow zero gas to flow until samples are analyzed.

If the proper meter readings are not achieved by following the above procedure, internal electronic adjustments may be required. The zero and span calibration knobs on the front of the instrument are "fine" adjustments. The detector in the analyzer may drift enough to render these knobs useless. When this happens, coarse adjustments need to be made inside the unit.

The occasion may also arise when samples of a higher concentration range need to be measured. The instrument may have to be re-spanned to avoid off-scale readings. The solid state amplifier of the analyzer becomes saturated above about 25 percent of full scale. The output then becomes flat and very non-linear. Re-spanning is basically an electrical compression of the output signal to the amplifier. The coarse adjustment may be used to produce a meter reading of one half or one quarter of the original setting for the span gas. This should greatly increase the linear measurable range of the

instrument. Of course, this is done by sacrificing some sensitivity at the lower concentration range which may have a detrimental effect on blank determinations.

If a decrease in sensitivity of the infrared analyzer is noted, a complete realignment of the optics may be necessary. A step-by-step procedure for both re-spanning and complete realignment are included in the Lira Model 303 instruction manual. The procedures appear tedious at first but are well covered in the manual. Re-spanning the instrument is part of the complete alignment procedure. The steps necessary for re-spanning are found on pages 2-10 of the manual. The attenuator and resistor 53 are easily adjusted with a small jeweler's screwdriver. Usually, an adjustment of the span control will change the zero setting, thus requiring re-zeroing. Do not attempt to realign the instrument before reading and understanding the entire procedure.

To complete the instrument calibration, turn on the electronic integrator and adjust the zero baseline with nitrogen gas flowing. With the analyzer meter on zero, adjust the zero control until integration occurs slowly. Back off the setting to the point where integration stops. Press the "PRINT" button on the integrator to check the response of the electronic printer. The printer output should agree with the digital display of the integrator.

INSTRUMENT OPERATION

Operation of the Total Carbon System is not complicated, but does require some practice. This report, in conjunction with the helpful illustrations in the Procedure and Operation manual, should benefit anyone involved with the Total Carbon System. The precision of the analysis will improve as the operator becomes more familiar with the technique.

Operation of Purging and Sealing Unit

The purging and sealing unit requires the most practice in order to become proficient in its operation. The power to the catalyst heater should be turned on one to two hours before sealing samples. When the pyrometer indicates that a temperature of 450°C has been achieved, open the main valve of the oxygen cylinder. Adjust the pressure regulator to 20 p.s.i. Allow fifteen minutes for the oxygen to purge the catalyst. Make certain that the purge tubes and cones have been carefully cleaned.

1. Begin purging and sealing samples by placing the first sample in the purging position.
2. Insert a purge tube through a purge cone and then into the ampule. Extend the tube all the way to the bottom of the ampule. Be sure that the tip of the purge cone sits down into the neck of the ampule to guard against contamination.
3. Each ten milliliter ampule must be purged for exactly six minutes to remove inorganic components. Part of the precision of the technique is determined by how closely

this time interval is observed for each sample. A stop watch is very helpful for this purpose.

4. Start the stop watch as purging is begun on the first sample. It takes approximately forty seconds to move an ampule from the purging position to the clamping assembly, seal the neck, remove the ampule, and place another sample in the vacated purging position. It is advantageous to establish a smooth flowing, sequential system of purging and sealing vials. This can be accomplished by placing an ampule in the purging position and inserting the purge tube every forty seconds. Begin with the purging position to the operator's right. Once the sequence is started, it should not be interrupted until all the vials have been sealed.

5. After forty seconds, place sample number two in the purging position to the left of sample number one. Insert the purge tube.

6. Continue this procedure until all eight purging positions are occupied. Approximately one minute should remain before the first ampule is ready to be sealed.

7. To ignite the microburner:

   Open the main valve on the propane tank.

   Open the propane toggle valve on the front of the ampule sealing unit.

   Ignite the propane at the microburner. Allow the flame to reach maximum intensity. Adjust the propane flow with the microburner adjust valve to produce a

flame about two to three inches high.

Open the oxygen toggle valve slowly. Note that if the propane flame is not allowed to reach its maximum intensity before introducing the oxygen, an oxygen-rich mixture will be produced. This will result in a small, but sharp, explosion!

Carefully adjust the oxygen flow valve to produce neutral flame tips of about 1/16 inch in length. Flame adjustment is critical and will be discussed further in regard to production of a properly sealed ampule.

8. After six minutes of purging, remove the first ampule from the purge position. Handle the ampule in such a way that the purge tube and cone remain in the neck of the vial.
9. Secure the purge tube to the small rubber holder over the clamping assembly. The point of attachment should be approximately one inch below the Teflon^{R} purge line.
10. Place the ampule in the clamping assembly. Position the vial in such a way that the top of the neck is flush with the upper jaw of the clamp.
11. Raise the base socket while pressing the ampule holding lever with the thumb.
12. Place the base of the ampule into position and release the lever. At this point the entire assembly should be self-supporting. The bottom of the purge tube should be even with the underside of the clamping assembly and just below the apex of the purge cone.

13. Move the microburner into a position which focuses all four jets on the neck of the ampule. Hold the base socket rod, with the thumb and two forefingers, just above the plexiglass support member. When the thin glass neck reaches the molten state, pull the ampule down about 3/8 inch. This is easily accomplished by lowering the base socket rod until the index finger rests on the support member. Thus the rod is lowered by a distance approximately equal to the diameter of the middle finger. By using this technique, ampules may be pulled down the same distance repeatedly. This movement decreases the diameter of the neck. Twist the rod with the thumb and index finger several times while maintaining the height of the ampule. The twisting motion completes the sealing process.
14. Remove the microburner as soon as possible and lower the ampule until the base socket rests on the plexiglass support.
15. Insert the purge tube and cone into another ampule and place it in the purging position.
16. Remove the sealed ampule from the base socket. The ampule may be placed directly into the oxidizing rack after allowing it to cool for a few seconds.
17. Open the clamping assembly and catch the <u>hot</u> ampule tip in a glass beaker.
18. Do not lose track of the ampules in the purging rack. By replacing every sealed ampule with an unpurged

vial in the purging rack, a continuous cycle may be established. The ampule sealing technique will require some practice to achieve properly sealed ampules. Refer to the illustrations for the criterion of a correctly sealed ampule (pp. 22 and 23).

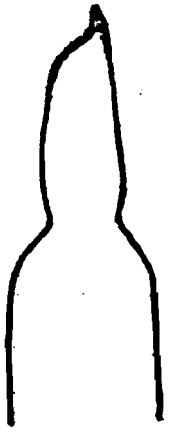
To turn off the sealing unit:

1. Close the oxygen toggle valve.
2. Allow the propane flame to stabilize.
3. Close the propane toggle valve.
4. Turn off the main valve on the propane bottle.
5. Close the valve on the oxygen cylinder.
6. Turn off the catalyst heater.

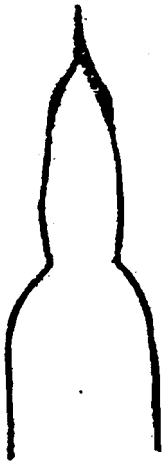
CRITERION FOR A PROPERLY SEALED AMPULE

Sealed Glass Ampules

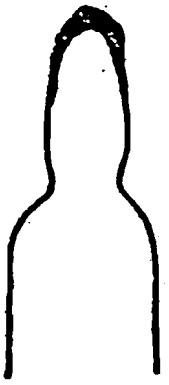
"A" - This ampule was sealed with a very hot flame and was not drawn down far enough prior to twisting the ampule. The resulting seal is very difficult to break in the breaking assembly. Basically, this ampule has a thickened glass top and is symmetrically round with respect to the vertical axis.



"B" - The seal on this ampule is one that is easily broken in the ampule breaking assembly. This seal is also durable and can be handled without breaking. Notice the glass on the top of this ampule was not thickened during sealing and is slightly non-symmetrical about the vertical axis resulting in an easily broken ampule.



"C" - This ampule seal has a long, sharp and easily broken top. Although it will break easily in the ampule breaking assembly this seal is easily broken during handling either during the oxidation phase or when the ampule is placed in the breaking assembly.



"D" - This ampule has a shortened neck because the neck of the ampule was clamped improperly during the sealing operation. Also the glass at the top of the ampule is thickened making it almost impossible to break this ampule in the breaking assembly.

The temperature of the flame and operator proficiency are very important in obtaining ampules of type "B" on the preceding page. Prior to sealing ampules with sample water, the operator should practice sealing ampules until proficiency is obtained in making a seal such as "B" which will break easily when placed in the ampule breaking assembly. Ampules that are improperly sealed will be very difficult to break causing bending of the cutters on the cutter plunger of the breaking assembly or be easily broken during handling of the ampule.

Taken directly from the Oceanography International Corp. (OIC) Instruction and Procedures Manual

Oxidation of Organic Matter in Sealed Ampules

The conversion of organic matter to carbon dioxide is accomplished at elevated temperatures. Quantitative oxidation of organic compounds by persulfate may be achieved by placing the sealed ampules in an autoclave set at 130 degrees centigrade for a period of at least four hours.

If an autoclave is not available, a Pressure Vessel, catalogue No. 0512VZ, may be purchased from Oceanography International Corp. The pressure vessel may be used to provide an external water vapor pressure equal to the vapor pressure within the sealed ampule during the oxidation process. The vessel is constructed of heavy gauge steel with a six inch inside diameter. Eighty-six 10 ml ampules may be accommodated in two racks provided with the vessel. A silicone rubber o-ring and a heavy steel cover with six 3/8 inch bolts are used to seal the container. Heat for the vessel is provided by any dry oven with a thermostat capable of maintaining a temperature of 175 ± 5 degrees centigrade.

The pressure vessel is simple to use, but some very important points must be observed. The least complicated method of placing the ampules in the metal racks is one at a time as they are sealed. It is imperative that a record be kept identifying each ampule to its corresponding numerical rack position. There is no reliable method of marking the ampules. Record pads may be purchased with the Carbon System.

Prepare the pressure vessel for use as follows:

1. Place the filled racks in the pressure vessel. One is marked upper, the other, lower, depicting their position in the vessel.
2. Add 1500 ml of water to the vessel. Never fill the vessel with water. This could cause extreme pressure within the vessel. The proper internal pressure, at 175° centigrade, should be approximately 115 pounds per square inch.
3. Be sure the o-ring is clean and add a light coat of silicone grease.
4. Place the cover on and tighten the bolts finger tight. Use a wrench to secure the bolts one-half turn at a time. The cover should be tightened evenly by turning alternate bolts.
5. Place the vessel in the oven, set at 175° centigrade, for sixteen to twenty-four hours.
6. Carefully remove the pressure vessel from the oven. Remember, it is very hot! Allow it to cool, unassisted, to room temperature. This will require at least four hours.
7. Loosen all bolts and be sure to relieve any internal pressure before completely removing the bolts.
8. Remove the ampules, dry them, and place them in the numbered storage racks for analysis using the ampule analyzing unit.
9. The silicone o-ring should be checked before each use

and should be replaced at any sign of excessive damage. The normal lifespan of the o-ring is fifteen or twenty digestions.

10. Dry the vessel before storage to minimize rust.

Operation of Ampule Analyzing Unit

Operation of the ampule analyzing unit is less complicated than the purging and sealing unit. After the instrument has been set up and the zero and span adjustments have been made, the operating technique is quite routine. The main consideration is to maintain the proper gas flow rates at all times.

To begin operation of the ampule analyzing unit:

1. Place a gray, plastic adaptor on the ampule to be analyzed. The small opening should face upward.
2. Slide a gum rubber seal over the neck of the ampule and down to the adaptor.
3. Place the ampule in the breaking assembly. Pull the plunger and purge tube up high enough to avoid any interference with the ampule as it is tightened into place. Check to see that the gum rubber seal is well seated in the glass barrel. Tighten the threaded elevator enough to assure a good seal, but not so much as to break the ampule prematurely.
4. Push the stainless steel purge tube down near the top of the ampule.
5. Move the two-way valve in to the "IR BY-PASS" position.
6. Open the zero gas and flow toggle valves. Check for a purging rate of approximately 200 ml/min or 13 on the rotometer.
7. Purge for at least ten seconds. This removes any carbon dioxide from the breaking assembly or the drying

system by way of an exit port in the rear of the cabinet.

8. Pull the two-way valve out to the "IR ANALYZE" position. This causes the carrier gas and the carbon dioxide to flow to the analyzer.
9. Check for a flow rate of 200 ml/min or 13 on the rotometer.
10. Place the FLOW toggle valve in the "OFF" position.
11. Allow the flow rate to drop to zero while checking to see that the purge tube is clear of the plunger cutters.
12. Crush the top of the ampule by applying pressure to and turning the handle of the plunger. A properly sealed ampule should break without too much difficulty.
13. Pull the plunger up approximately 1/2 inch from the top of the ampule.
14. Push the purge tube to within 1/2 inch of the ampule bottom.
15. Open the flow toggle valve and, again, check for a flow of 13. Two or three seconds are required for the flow to reach a steady state. After the initial gas surge and subsequent plug of water has settled back into the ampule, push the purge tube to within 1/8 inch of the bottom of the ampule.
16. Move the two-way valve back in to the "BY-PASS" position after the peak has been formed and the indicator on the analog meter has returned to exactly 5 percent. This is purely a time-saving step. The valve could be changed as the signal returns to baseline. However, there is no loss of accuracy or precision as long as the valve is changed at exactly 5 percent for the

standards, blanks and samples.

17. Remove the broken ampule. Be careful of crushed glass.
18. Print the integrator reading by pressing the "PRINT" button. If the integrator is operated in the "AUTO-ZERO" mode, the display is automatically cleared as the reading is printed.
19. Prepare another ampule with a plastic adaptor and gum rubber seal and proceed as before.

The flow rate of the carrier gas is extremely critical. The peak area of a sample will vary greatly if the rate is changed. Even a slight obstruction of any gas line may change the rate of flow. To avoid any blockage, the entire breaking assembly should be dismantled and cleaned of glass particles after twenty to thirty samples have been analyzed. The drying tubes also represent a potential gas flow problem. As water vapor passes through the system to the tubes, the drying agent becomes exhausted. This is evident as the magnesium perchlorate in the lower part of the primary tube becomes semi-liquid. This will cause a decrease in the gas flow rate. When this occurs, the second tube should be moved to the primary position and a freshly packed tube placed in the second position. Any water which collects in the vapor trap should be removed between samples. The water vapor problem can be reduced by pulling the plunger up approximately 1/2 inch after breaking the top of the ampule. When the purge tube is fully extended into the ampule, it will reach to within 1/2 to 3/4 inch from the bottom of the ampule. This extra step should be done with care to avoid any loss of

carbon dioxide. After opening the flow valve and the initial surge of gas has passed, the plunger and purge tube should be pushed down to the proper position. It is advisable to have several freshly packed drying tubes prepared before starting analysis of a series of samples. If water vapor should find its way to the detector, significant damage may be incurred.

Each time an analysis is begun, it is recommended that from three to five trial ampules be run to establish a water vapor equilibrium in the system.

OPTIMIZATION OF TECHNIQUE

This method for organic carbon analysis is quite straightforward, but several factors require refinement for optimum results. The quality of the reagents used in the procedure is very important. Organic compounds are commonly found almost everywhere and are a major source of contamination. It is impossible to obtain reagents totally free of organic contaminants. The alternative is to reduce the effect of the interference and assess its contribution to the result.

The reagent capable of contributing the most interference from organic carbon contamination is water. The standards are prepared using water as the solvent. The potassium persulfate is dissolved in water and at times, water is added to the sample. Thus, water with a high organic carbon content may produce a spurious signal rendering the method useless.

Eliminating the organic carbon components from water is a difficult task. The various types of water readily available in the laboratory result from diverse cleaning techniques. The water from all the different sources was analyzed to determine that which exhibited the lowest organic carbon level. The tap water in the laboratory is processed by the Harbor Branch Foundation facility with a reverse osmosis technique. This water is further treated by a second reverse osmosis system and passed through a deionization resin bed. As a final step, this water is cleaned by a Milli-Q System^{R}

using three ion exchange cartridges. Water from each cleaning stage was analyzed. Furthermore, tap water from the men's room was tested because of its reduced exposure to polyvinyl chloride (P.V.C.) pipes. The solvent used on the recently connected pipes was a suspected contaminant. The final source of water was in the stockroom. Product water from the second reverse osmosis system may be collected before it is passed through the deionization resin bed. Organic carbon levels found in the various types of water appear in Table 1 expressed as reagent blank peak area. The data shows that the water collected after the second reverse osmosis treatment, but before the deionization bed, contains the least organic carbon. Tap water in the men's room is also quite free of organic contaminants. In both cases, the water is not exposed to as much of the P.V.C. solvent used to connect the pipes leading to the laboratory. Water passed through the deionization resin bed is high in organic carbon levels because of the extra exposure to the P.V.C. solvent and due to organic coating of the resin. Further study showed the contamination level of this water to be a function of the age of the resin bed. Large amounts of organics are washed from a freshly charged deionization bed.

WATER CLEANING METHODS

In order to improve the analytical technique by lowering the carbon level in the reagent blank, various water cleaning methods were examined. The first technique attempted was distillation. This effort resulted in little success.

TABLE 1

Organic Carbon Levels in 5 Types of Water

Data shown is from blanks with reagents and the designated water expressed as peak area units.

WATER TYPE	PEAK AREA	COEFFICIENT OF VARIATION
Tap water (in lab)	3362	10%
R.O. water (after D.I.)	3720	1%
R.O. water (before D.I.)	1443	11%
Milli-Q system	3181	16%
Mens Room (tap water)	1462	18%

Apparently, boiling and subsequent condensation of water does not remove the more volatile organics which are carried with the distillation process. Furthermore, the increased handling necessary to carry out the process presents an excellent opportunity for increased contamination.

Filtration through activated charcoal was also considered as a cleaning method. Reverse osmosis water (before deionization) was passed through a cartridge containing activated charcoal. After the cartridge has been rinsed with three liters of water, an aliquot was collected for analysis. Reagent blanks were prepared from water before and after passage through the charcoal. This method also lead to disappointing results. The water which passed through the charcoal was over three times higher in organic carbon than the untreated water. The activated charcoal was obviously high in loosely bonded organics which were washed free by the water.

The results of both the distillation and activated charcoal experiments are shown in Table 2.

After achieving little success with the above methods, ultraviolet irradiation was considered as a cleaning technique. According to Armstrong, et al (1966) exposure of organic carbon to ultraviolet light is a very efficient oxidation technique.

It was theorized that if water was exposed to the ultraviolet light, the resulting carbon dioxide could be displaced by bubblings with an inert gas.

TABLE 2

Organic Carbon Levels Before and After Treatment
by Distillation and Activated Charcoal Filtration

Data shown is from blanks with reagents and the designated water expressed as peak area units.

TREATMENT	PEAK AREA	COEFFICIENT OF VARIATION
Before Distillation	1240	17%
After Distillation	1828	8%
Before Activated Charcoal	1334	5%
After Activated Charcoal	4275	9%

A high intensity (450 watt) ultraviolet lamp with ancillary equipment was purchased from Ace Glassware Corporation.

The first experiment with ultraviolet irradiation was designed to not only assess the merit of the technique but also to study the effect of adding additional oxidizers to the water. Four separate aliquots of water were prepared for irradiation. The first was simply R.O. water taken before passage through the ion exchange column. The next lot contained the same R.O. water with the addition of enough 30% hydrogen peroxide to achieve a 0.3% solution. The third batch was made up of the R.O. water plus an amount of potassium persulfate solution yielding a final solution of about 1×10^{-2} M $K_2S_2O_8$. The final sample consisted of the R.O. water along with both oxidizers. All four lots were irradiated at the same time for a time period of one hour. After cooling, a set of reagent blanks was prepared from each solution. In addition to the above, reagent blanks were prepared from the R.O. water without any ultraviolet treatment. The results of the above study appear in Table 3 expressed as peak area units.

The data clearly proves ultraviolet irradiation to be an efficient method of organic oxidation. While the unexposed parcel of water contained the typically high level of organic carbon compounds, the same water exposed to the U.V. light for a period of one hour contained less than half the amount of organic carbon.

TABLE 3

The Effect of U.V. Irradiation on
Water Used for Reagent Blanks

SAMPLE	PEAK AREA	STD. DEV.	CO. VAR.
Reagent blank with R.O. water plus reagents with no U.V.	3122	181	6.0%
Reagent blank with R.O. water plus reagents with U.V.	1326	139	10.4%
Reagent blank with R.O. water plus reagents and 1ml of 30% H_2O_2 with U.V.	1890	216	11.5%
Reagent blank with R.O. water plus reagents and 5ml of $K_2S_2O_8$ with U.V.	1291	210	16.2%
Reagent blank with R.O. water plus reagents and both H_2O_2 and $K_2S_2O_8$ with U.V.	1434	161	11.2%

The attempt to improve the efficiency of the technique by the addition of oxidizing agents produced questionable results. The set of blanks with peroxide added exhibited a peak area of about 50% higher than that of R.O. water with no oxidizer. The ultraviolet treatment was still effective but apparently the peroxide added a contaminant which was not removed by the U.V. exposure. The addition of potassium persulfate with the U.V. treatment was more successful, producing a slightly lower mean peak area than pure R.O. water. However, the coefficient of variation is 6% higher and the small decrease in peak area is not significant. Finally, the blank with both peroxide and potassium persulfate was also higher than that of pure R.O. water. The only explanation for this is contamination by the reagents.

After having established the merit of the U.V. irradiation as a cleaning technique, a further study was conducted to assess the optimum exposure time. The results of this study indicated that a two-hour irradiation period produced the lowest achievable reagent blank. Although longer time periods were attempted, apparently a two-hour exposure is sufficient to oxidize all the material which is susceptible to U.V. irradiation.

The conclusion based on the study of the various water cleaning techniques is that laboratory water may be cleaned sufficiently for use in the total organic carbon method. Although the U.V. irradiation technique produces the desired results, it is a costly and expensive part of the analysis procedure.

Commercially Available Water

After spending a great deal of effort to produce water with an acceptable level of organic material, a possible "off the shelf" product was discovered. The J.T. Baker Chemical Co. announced a new product called H.P.L.C. Water. This Baker "reagent" was advertized to be "free of organic impurities." The potential time saved by the use of a reliable, ready-to-use, "organic-free" water source made investigation into this product justifiable.

The details of the study assessing the applicability of H.P.L.C. Water to organic carbon analysis are shown by Peterson and Montgomery (1979). To summarize the findings of that paper, H.P.L.C. Water was found to be unacceptable for use in organic carbon analysis. The quality control technique used in the production of the Baker water is limited to assessment of the impurities at the 254 nm wavelength used in High Performance Liquid Chromatography.

As a result of the above described study and resultant paper, the J.T. Baker Chemical Co. has marketed another new product called Baker Instra-Analyzed Water. This water is processed in the same manner as H.P.L.C. Water with an additional Q.C. analysis to determine the amount of TOC. The maximum allowable level is 100 ppb, with the assay included on the bottle. This new product seems to meet the requirements of a low organic level, ready-to-use water for the TOC analysis. In spite of its high cost, the convenience and time saved justifies its use.

Potassium persulfate

Reagent grade potassium persulfate is used to enhance the oxidation of organic carbon material to CO_2. A super-saturated solution of $K_2S_2O_8$ should be prepared by adding 5.0 grams to 100 mls of "low organic" water.

In the interest of lowering the reagent blank, the effect of recrystalizing the $K_2S_2O_8$ was studied. The details of the study are shown in the TOC lab notebook. The results were disappointing. The reagent blank produced using the re-crystalized persulfate was about 25% higher and exhibited higher variation than the reagent grade potassium persulfate. Apparently, the increased handling and filtration necessary for the recrystalization lends itself to contamination. The basic premise of recrystalization as a reagent cleaning technique has been established. However, in order to make it applicable to this situation, the procedure will need to be "cleaned up" to avoid the overshadowing contamination problem.

A "low-nitrogen" lot of potassium persulfate was also tried as the oxidant. The resultant reagent blank was not discernibly different from that of regular reagent grade $K_2S_2O_8$.

Phosphoric Acid

A 6% solution of 85% phosphoric acid is used to rid the sample of inorganic carbonate species. The phosphoric acid produces a very small percentage of the reagent blank value.

Standards and Blanks

Analytical quality sucrose ($C_{12}H_{22}O_{11}$) may be used as an organic carbon source for the production of a calibration curve. One gram of sucrose contains 0.4211 gram of organic carbon. A series of various concentrations of sucrose solutions may be treated by the wet digestion technique. Each concentration of sucrose is oxidized to CO_2 quantitatively, being proportional to the original amount of organic carbon in the solution.

The instrument operation manual describes a method for the preparation of a calibration curve. The six concentrations of sucrose produced by the prescribed method cover the entire range of the CO_2 detector when calibrated to the recommended sensitivity.

Refer to the manual for details of the calibration curve preparation yielding the following concentrations:

1. 2.0 mg Carbon/liter
2. 1.5 mg Carbon/liter
3. 1.0 mg Carbon/liter
4. 0.8 mg Carbon/liter
5. 0.5 mg Carbon/liter
6. 0.2 mg Carbon/liter

Due to the fact that sample size is variable depending on the level of organic material, it is more convenient to express the calibration curve in terms of weight. Final concentrations of samples may then be computed based on the sample volume. The volume of the standard solutions to be analyzed is constant however. Based on a 10 ml aliquot of the above standard

solutions, the calibration curve may be expressed in terms of weight as follows:

1. 0.020 mg Carbon
2. 0.015 mg Carbon
3. 0.010 mg Carbon
4. 0.008 mg Carbon
5. 0.005 mg Carbon
6. 0.002 mg Carbon

It should be obvious from the above section on "low organic water" that the water used in this technique is a precious commodity. In the interest of conserving the expensive solvent, this laboratory recommends the use of the following preparation scheme.

Stock solution:

Dissolve 0.11875 gram of sucrose in "low organic carbon" water and dilute to 500 mls.

Sub-standard #1:

Dilute 50 mls of stock solution to 500 mls.

Sub-standard #2:

Dilute 50 mls of sub-standard #1 to 500 mls.

Standard solutions:

1. Dilute to 100 mls of sub-standard #1 to 500 mls = 0.020 mg Carbon.
2. Dilute 75 mls of sub-standard #1 to 500 mls = 0.015 mg Carbon.
3. Use sub-standard #2, undiluted, as a solution containing 0.010 mg Carbon.

4. Dilute 160 mls of sub-standard #2 to 200 mls = 0.008 mg Carbon.
5. Dilute 100 mls of sub-standard #2 to 200 mls = 0.005 mg Carbon.
6. Dilute 40 mls of sub-standard #2 to 200 mls = 0.002 mg Carbon.

Be sure to use the good quality water for all of the dilutions.

All glassware and apparatus needed to prepare the above standard solutions should be thoroughly cleaned by:

1. Soaking at 60°C in a 1.0% Triton-X solution for two days.
2. Soaking in a 3:1 solution of nitric/hydrochloric acid for three hours.
3. Copious rinsing with good quality laboratory water and a final rinse with the "low-organic carbon" water.

All standard solutions, including the stock, should be prepared fresh on the day which samples are to be sealed and digested.

Standards are prepared for sealing and digestion by pipetting 10 ml of the standard solution into a precombusted ampule along with the potassium persulfate and phosphoric acid. At least three replicates should be prepared for each standard. The details of sample, standard, and reagent addition to the glass ampules will be described further in the section covering sample preparation.

Reagent blanks should be prepared along with the standards. The blank solutions consist of the reagents along with 10 mls of the water used to prepare the standards. A minimum of ten reagent blanks should be prepared with each analysis.

A more simple, alternate calibration curve is described in the Oceanography International Instrument Operation Manual. This more rapid method uses sodium carbonate to liberate CO_2 for calibration of the detector. The sodium carbonate method should be used only after it has been carefully cross-standardized with the above ampule technique using sucrose.

This laboratory did not find an acceptable agreement between the two methods of preparing a calibration curve. Therefore, the sodium carbonate method is not recommended and will not be described in this report. Complete details of the technique may be found in the operation manual or the T.O.C. laboratory notebook. It is the opinion of this laboratory that the ampule calibration method using sucrose is more valid than the rapid sodium carbonate method. The sucrose must be digested in the same manner as the organic carbon in the samples. Thus, the sucrose standard curve results in an indication of the efficiency of the reagents and the heating process. The rapid method simply liberates CO_2 from the breakdown of sodium carbonate in an acid medium. While this is quick, it is not indicative of the technique.

In an effort to further improve the overall T.O.C. analysis, recovery studies should be conducted using primary standards as the source of organic carbon. Potassium

biphthalate or acetanilide may both be used as reference standards for determination of organic carbon. Other compounds of higher order bonding structures may also be of interest as they would more closely typify the organic material found in actual samples.

SAMPLE COLLECTION, STORAGE AND ANALYSIS

Samples to be analyzed for organic carbon may be collected using various techniques. Traditional collection apparatus may be used provided the sampling vessel is clean and is not used to store the sample. This laboratory is currently using porous teflon, vacuum assisted samplers to collect anoxic interstitial water for organic carbon determination.

Samples should be filtered immediately upon collection to separate the dissolved from the particulate organic fraction. Gelman glass fiber filters are recommended for the filtration. The particulate organic carbon determination will be discussed further in another section of this report.

Samples should be stored in tightly sealed, glass containers. Keep the sample cool after collection and freeze as soon as possible. Do not remove samples from the freezer until they are to be sealed and digested. After digestion, samples may be stored indefinitely in the sealed ampules until they are analyzed.

Clean the storage containers in the same manner as previously described for glassware used to prepare standards.

Sample Preparation

All ampules should be precombusted to eliminate contamination. Oceanography International Corp. supplies precombusted glass ampules for the Total Carbon System. This laboratory feels that it is more cost effective to purchase these ampules than to devise an in-house cleaning technique.

Ampules are supplied with aluminum foil wrapped tops to assure cleanliness. When preparing samples, remove the foil and place an inverted plastic sample cup over the vial's neck. Place the required number of ampules in the rectangular, numbered rack. An accurate record of the ampule position numbers must be kept through the entire analysis. There is no quick, reliable method of marking the ampules.

Samples, standards, and blanks are prepared for sealing and digestion by following these steps.

1. Add 1.0 ml of the saturated potassium persulfate solution to each vial using a repeating pipette.
2. Add 0.25 ml of 6% phosphoric acid to each vial using a repeating pipette. (Recap vials between each addition).
3. Add the appropriate solution for samples, standards, or blanks as described below.

Samples - Use the optimum volume depending on the expected concentration and add "clean" water when necessary. This will be discussed further in the section describing optimization of sample volume.

Standards - Add 10 ml of freshly prepared standard solutions.

Blanks - Add 10 ml of the "clean" water used to prepare standards and dilute samples when necessary.

In order to reduce contamination follow these recommendations:

1. Always do all sample handling under a laminar hood and keep all solutions and ampules covered whenever possible.
2. Do not touch the neck of the ampules.
3. Clean the purge tubes on sealing unit in boiling water before each use.
4. Prepare the lowest concentration of samples and standards first.

Optimization of sample volume

Sample size must be experimentally determined in order to have the CO_2 level in the operational range of the detector. Due to the kinetics of the CO_2 degassing, no less than 5 mls of solution should be placed in an ampule. If a sample contains an organic carbon level requiring an aliquot of less than 5 ml, an appropriate volume of organic-free water must be added.

The following guidelines may be used to approximate sample size.

Expected Carbon Range, mg C/l	Amount of Sample	Amount of organic-free water to add
0-2	10 ml	0
2-10	5 ml	0
10-30	2 ml	3 ml
30-50	1 ml	4 ml
50-100	0.5 ml	5 ml
100-250	0.25 ml	5 ml

Expected Carbon Range, mg C/l	Amount of Sample	Amount of organic-free water to add
250-500	0.10 ml	5 ml
500 and above	0.05 ml	5 ml

Always use the largest sample size which produces a well defined peak without overwhelming the detector.

PARTICULATE ORGANIC CARBON

Samples requiring determination of particulate organic carbon must be filtered to separate the dissolved from the particulate fraction. The filter pad with the suspended material is placed directly into an ampule for digestion.

Gelman^{R} Type A glass fiber filters or an equivalent non-organic type are recommended for filtration. Use filters having a 25 mm diameter. Large filter pads will not fit into an ampule for digestion.

Precombust the filters to remove volatile organics as follows:

1. Place filters, individually, in a layer on a screen wire rack without overlap. Note: The rack must be pre-cleaned by heating to 550°C for two hours.
2. Insert rack with filters in a muffle furnace and heat to 400°C for a period of two hours.
3. Allow filters to cool and then place them in a clean, air-tight container.

Always handle the filter pads with clean forceps from the time they are removed from the original package until they are placed in the ampules for digestion.

A 25 mm, plastic, in-line filter holder is available from Gelman^{R}. This holder works well with a peristaltic pump to accomplish filtration as part of the sampling scheme.

The size of the aliquot of water to be filtered is an important consideration in order to achieve a representative particulate sample. The aliquot size varies from 100 ml for

estuarine water to 1000 ml for open ocean water depending on the organic material content. As a rule of thumb, filter a sufficient quantity of sample to produce a visible particle load on the filter pad. A few filters may need to be sacrificed for this trial and error procedure.

After filtration, prepare the filter pads for digestion as follows:

1. Remove the filter pad from the holder with clean forceps.
2. Fold the filter in half using two pair of forceps.
3. Place on a clean piece of aluminum foil and roll the filter pad into a small cylinder using the forceps.
4. Insert the rolled filter into an ampule to which persulfate has been added.
5. Add 0.25 ml of 6% phosphoric acid.
6. Add 5.0 ml of "low organic carbon" water.

Note: This laboratory deviates from the order in which solutions are added to the ampules prescribed by the OIC manual. The manual recommends that the phosphoric acid be added last. We feel that due to the small volume added, any acid sticking to the sides of the ampule would have significant consequences. The addition of 5.0 ml of "clean" water <u>last</u> helps to wash the reagents down into the ampule.

7. Purge and seal the ampules as previously described.
8. After digestion in the pressure vessel the samples may be analyzed in the same manner as the dissolved fraction.
9. Prepare a standard calibration curve and reagent blanks with each set of samples to be analyzed.

Preparation of reagent blanks for POC

Reagent blanks for the particulate organic carbon determination must reflect the contribution to the CO_2 signal from the filter pad. The filter must be handled in exactly the same manner as those used for filtration. Load the filters to be used as reagent blanks into filter holders. Wet the filter with "clean" water. Insert the "blank filters" into ampules, add reagents, and treat exactly as samples.

DATA REDUCTION

The raw data output from the Total Carbon System is in the form of an integrated peak area. The digital display is indicative of the CO_2 liberated from the vial which is proportional to the organic carbon originally in the sample.

Record all data on log sheets along with all pertinent information such as sample description, sample volume, date, etc. Data may also be recorded on the digital printer for cross reference.

A typical analysis scheme should consist of the following:

1. A standard calibration curve consisting of three replicates of the six concentrations previously described.
2. A series of reagent blanks containing increasing amounts of low organic carbon water from 2 ml to 10 ml. Specifically, prepare two each of water volumes 2 ml, 4 ml, 6 ml, and 8 ml. Prepare 5 reagent blanks containing 10 ml of low organic carbon water. The purpose of this standard addition like treatment of the reagent blank is to delineate the contribution of the various components of the blank. By extrapolating to zero using a linear least squares fit, the signal generated by reagents only may be determined. Subtraction of this value from the 10 ml volume reagent blank depicts the portion of the reagent blank generated by the water. Division by 10 finally yields the amount of interference produced per ml of water.

3. Three replicates of each sample to be analyzed.

Reduce the raw data to achieve final concentration values for samples in the following manner:

1. Compute the mean peak area and standard deviation of the 10 ml volume reagent blanks.
2. Subtract the mean 10 ml volume reagent blank value from all of the standard calibration curve.
3. Perform a linear regression analysis on the standard calibration curve.
4. Perform a linear regression analysis on the full series of reagent blanks. The intercept generated by the regression represents the reagent contribution to the reagent blank. Subtract this value from the mean 10 ml volume reagent blank. This value divided by 10 represents the CO_2 signal generated by 1 ml of water used as a solvent and diluent.
5. Multiply the per ml water contribution of the reagent blank by the amount of low organic carbon water added to each sample. Add this value to the reagent contribution to determine the "actual blank" value to be subtracted from the samples.
6. Subtract the "actual blank" value determined in step #5 from the integrated peak area value of each sample replicate.
7. Use the equation generated by the regression performed on the standard calibration curve to compute the milligrams of carbon in each sample replicate.

8. Multiply the concentration of each sample replicate by (1000 divided by the sample volume in mℓ) to express concentration as mg C/Liter.

9. Compute the mean concentration, standard deviation and coefficient of variation of each of the samples.

A computer program was devised to accomplish the data reduction. The program is named PDOC. The algorithm follows the same logical order as described above. In addition, a Student t-distribution is performed to compare each new set of blanks to all previous blanks. Values for t are determined at the 95% confidence level.

In an effort to maintain a running evaluation of the method, a cumulative sum of the standard calibration slopes as described by Montgomery (1976) is included in PDOC.

The minimum detectable concentration (M.D.C.) is determined and updated with each analysis as follows:

$$\text{M.D.C.} = \frac{t\ (\text{std. dev. of blank})}{\text{slope}}$$

where: t is the Student t value for n-2 (degrees of freedom) std. dev. of blank is the standard deviation of the overall mean of all blanks run to date, slope is the slope of the standard calibration curve generated with the data set presently being reduced.

All reagent blank values expressed in peak area, calibration curve slopes expressed as mg C vs peak area and MDC expressed as mg C/Liter are recorded in data files.

Final concentration values for the samples (mg C/Liter) are stored in master file called CMASTER. This virtual file

contains all of the chemistry data from interstitial water collected weekly for one year.

Please refer to the appendix for an example demonstrating the use of the PDOC computer program.

AUTOMATION OF INSTRUMENT

In the interest of decreasing operator devoted analysis time, this laboratory has attempted to automate the ampule analyzing unit. Operation of the original unit is quite regimented and lends itself very well to mechanical automation. For example, open valve, close valve, break vial, close switch, and so on.

With the cooperation of the Harbor Branch Foundation R&D department, a stepwise flowchart of the analysis scheme was devised. This was further developed into a design of the actual circuitry and hardware.

Primary considerations in the design were concerned with the maintenance of our high standard of precision and accuracy. This was accomplished by succinct, mechanical steps, giving careful attention to the achievement of a gas-tight system with a constant flow rate.

Operation of the automated system is quite simple. The drying tubes and detector on the original unit are used in the automated system. To begin operation, insert freshly packed drying tubes and calibrate the I.R. detector as described previously. Insert approximately thirty ampules into the gray plastic adaptors and load them into the gravity feed slide. Turn on the electronic integrator and the digital printer. Turn the automated system switch on, then off again to move a vial into the proper position for initial start-up. Check the flow rate on the rotometer. Turn the automated system switch on to begin analysis. The system will break

the ampule, degas the CO_2, and check for proper gas flow rate. If the flow rate is incorrect, an alarm will sound alerting the operator. After the CO_2 peak has occurred and the integration stops, the printer is triggered and the electronic integrator is cleared for the next sample. The digital signal is printed and also simultaneously stored by a microprocessor. After the analysis, the data is transferred to a file on the main computer system where it may be accessed by the data reduction software.

The automated system has not been fully evaluated to date. Before routine use, the system must be rigorously tested to assure that its performance is equal to or above that of the original unit.

DISCUSSION

The method described here for the determination of total organic carbon works well in all natural waters. The technique is not plagued with interferences and has a fairly rapid sample throughput. By following the recommendations made in this report, an efficient, well controlled analysis program may be established and maintained.

The accuracy and precision of the method is well exemplified by the results of an interlaboratory calibration study conducted by the Department of Environmental Regulation.

This laboratory participated in the performance evaluation on 10/24/80. Two samples were analyzed for TOC by the above described technique.

The results were as follows:

Sample	Concentration	RSD*
#1	8.27 mg C/Liter	3.5%
#2	61.6 mg C/Liter	5.8%

The "expected" value for sample #1 as quoted by the D.E.R. was 8.08 mg C/Liter. Using this as the "true" value, the HBF value was +2.4% accurate. The "expected" value for sample #2 was 60.61 mg C/Liter. The HBF accuracy for this sample was +1.6%. Both values were well within the statistical boundaries of acceptable results using two times the standard deviation as a criterion.

*R.S.D. - relative standard deviation

$$\text{R.S.D.} = \frac{\text{standard deviation}}{\text{mean concentration}}$$

In order to further improve the technique, the recovery study using primary standards as discussed previously should be performed. Secondly, a rigorous study should be undertaken to determine the optimum storage conditions for samples prior to sealing and digestion.

Although the detection limit of the technique is quite acceptable (0.05 - 0.15 mg C/Liter) for the concentration ranges this laboratory is currently studying, continual attempts are made to improve the sensitivity.

APPENDIX

The computer program for the reduction of the raw DOC data is PDOC. Data, in the form of integrated peak area, must be entered from the computer terminal. To call the program, simply type RUN PDOC. The main consideration in executing the program is the order of data entry. Enter data in the following order.

Standards:

.002 mg C

"

"

.005 mg C

"

"

.008 mg C

"

"

.010 mg C

"

"

.015 mg C

"

"

.020 mg C

"

"

Reagent Blanks

2 ml

"

Standards: (Cont.)

4 ml

"

6 ml

"

8 ml

"

10 ml

"
"
"
"

Sample #1

"
"

Sample #2

"
"

etc.

The total number of data points to be entered must be supplied at the beginning of the program. If a data point is missing, enter 99999.

The following example depicts a typical data reduction using PDOC.

```
>RUN PDOC
ARE YOU ENTERING NEW DATA [Y/N]? Y
YOUR DATA WILL BE STORED IN VIRTUAL FILE DOC.DCM
ENTER THE NUMBER OF DATA POINTS TO BE STORED
? 67
ENTER THE DATA
 1 -? 2786
 2 -? 99999
 3 -? 2907
 4 -? 4228
 5 -? 3831
 6 -? 3982
 7 -? 5205
 8 -? 6026
 9 -? 5127
 10 -? 5867
 11 -? 5858
 12 -? 5554
 13 -? 7505
 14 -? 7533
 15 -? 7836
 16 -? 10108
 17 -? 9755
 18 -? 9711
 19 -? 789
 20 -? 99999
 21 -? 1075
 22 -? 945
 23 -? 1032
 24 -? 99999
 25 -? 1171
 26 -? 997
 27 -? 1187
 28 -? 1162
 29 -? 1353
 30 -? 1252
 31 -? 1268
 32 -? 4748
 33 -? 4123
 34 -? 4135
 35 -? 3441
 36 -? 99999
 37 -? 3728
 38 -? 2755
 39 -? 2487
 40 -? 2864
 41 -? 6268
 42 -? 6390
 43 -? 6256
 44 -? 2966
 45 -? 1953
 46 -? 2243
 47 -? 2599
 48 -? 2621
 49 -? 2635
 50 -? 2532
 51 -? 2500
 52 -? 2461
 53 -? 5158
 54 -? 5491
 55 -? 4898
 56 -? 4140
 57 -? 3720
 58 -? 3728
 59 -? 3858
 60 -? 3606
 61 -? 3675
 62 -? 6289
 63 -? 6142
 64 -? 5973
 65 -? 4685
 66 -? 4692
 67 -? 4789
```

```
ARE ANY CORRECTIONS NECESSARY
? NO
WOULD YOU LIKE A LISTING OF THE DATA
? YES
```

1	2	3	4	5
2786	99999	2907	4228	3831
6	7	8	9	10
3982	5205	6026	5127	5867
11	12	13	14	15
5858	5554	7505	7533	7836
16	17	18	19	20
10108	9755	9711	789	99999
21	22	23	24	25
1075	945	1032	99999	1171
26	27	28	29	30
997	1187	1162	1353	1252
31	32	33	34	35
1268	4748	4123	4135	3441
36	37	38	39	40
99999	3728	2755	2487	2864
41	42	43	44	45
6268	6390	6256	2966	1953
46	47	48	49	50
2243	2599	2621	2635	2532
51	52	53	54	55
2500	2461	5158	5491	4898
56	57	58	59	60
4140	3720	3728	3858	3606
61	62	63	64	65
3675	6289	6142	5973	4685
66	67	68	69	70
4692	4789	.301371E-14	.00978473	205009

```
WOULD YOU LIKE TO MAKE ADDITIONAL CORRECTIONS ?
? NO
ENTER 1 FOR DOC, 2 FOR POC? 1
ENTER THE WEEK NUMBER? 512
ENTER THE NUMBER OF SAMPLERS ? 12

***FOR SAMPLER NO.  1  ***
ENTER THE STATION NUMBER ? 1
ENTER THE SAMPLER NUMBER ? 1
ENTER SAMPLE VOLUME ? .5

***FOR SAMPLER NO.  2  ***
ENTER THE STATION NUMBER ? 1
ENTER THE SAMPLER NUMBER ? 2
ENTER SAMPLE VOLUME ? .5

***FOR SAMPLER NO.  3  ***
ENTER THE STATION NUMBER ? 1
ENTER THE SAMPLER NUMBER ? 3
ENTER SAMPLE VOLUME ? .5

***FOR SAMPLER NO.  4  ***
ENTER THE STATION NUMBER ? 1
ENTER THE SAMPLER NUMBER ? 4
ENTER SAMPLE VOLUME ? .5

***FOR SAMPLER NO.  5  ***
ENTER THE STATION NUMBER ? 1
ENTER THE SAMPLER NUMBER ? 5
ENTER SAMPLE VOLUME ? .5

***FOR SAMPLER NO.  6  ***
ENTER THE STATION NUMBER ? 1
ENTER THE SAMPLER NUMBER ? 6
ENTER SAMPLE VOLUME ? .5

***FOR SAMPLER NO.  7  ***
ENTER THE STATION NUMBER ? 1
ENTER THE SAMPLER NUMBER ? 7
ENTER SAMPLE VOLUME ? .5

***FOR SAMPLER NO.  8  ***
ENTER THE STATION NUMBER ? 1
ENTER THE SAMPLER NUMBER ? 8
ENTER SAMPLE VOLUME ? .5

***FOR SAMPLER NO.  9  ***
ENTER THE STATION NUMBER ? 2
ENTER THE SAMPLER NUMBER ? 11
ENTER SAMPLE VOLUME ? .5
```

```
***FOR SAMPLER NO.  10  ***
ENTER THE STATION NUMBER ? 2
ENTER THE SAMPLER NUMBER ? 12
ENTER SAMPLE VOLUME ? .5

***FOR SAMPLER NO.  11  ***
ENTER THE STATION NUMBER ? 1
ENTER THE SAMPLER NUMBER ? 13
ENTER SAMPLE VOLUME ? 2

***FOR SAMPLER NO.  12  ***
ENTER THE STATION NUMBER ? 2
ENTER THE SAMPLER NUMBER ? 15
ENTER SAMPLE VOLUME ? 2

**********************************************************************
STATISTICS FOR 10ML REAGENTS AND R.O. WATER
MEAN=  1244.4
S.D.=  74.9883
C.O.V.=  6.02606

T-TEST:OLD-NEW
              T CAL= -.0509643
              T TABLE=  1.986  D.O.F.=  93
NEW AND OLD BLANKS DO NOT DIFFER
**********************************************************************
RESULTS FOR STANDARD CURVE-BLANKS
LINEAR REGRESSION

ANOVA RESULTS
VAR SOURCE     DF            SS              MS              F
AMOUNG GRPS     5             .851741E 08     .170348E 08     231.506
LIN REG         1             .846672E 08     .846672E 08     668.081
DEV FROM REG    4             506928          126732          1.72231
WITHIN GRPS    11             809408          73582.5
TOTAL          16             .859836E 08

SLOPE =  380033               INTERCEPT =  883.429

**********************************************************************RESU
ENTER THE PROBABILITY LEVEL NUMBER FROM THE FOLLOWING LIST:
0=95%          1=90%          2=80%          PROB. LEVEL= ? 0
T=  2.056  FOR  27  DEG OF FREEDOM (MAX D.O.F.=120)
DELTA EQUALS  10011.9
DIFF BETWEEN MEAN AND EXP. SLOPES  20591.8
CUM. SUM -62755.9
DISTANCE  45.7781
CRITICAL HT.  229164
CUM. SUM IS WITHIN CONTROL RANGE
```

**********MIN
****
RESULTS FOR REAGENT BLANKS
LINEAR REGRESSION

ANOVA RESULTS

VAR SOURCE	DF	SS	MS	F
AMOUNG GRPS	4	220770	55192.5	7.18824
LIN REG	1	202373	202373	33.0006
DEV FROM REG	3	18397.2	6132.39	.798679
WITHIN GRPS	6	46069	7678.17	
TOTAL	10	266839		

SLOPE = 47.7581 INTERCEPT = 755.894

****
CONC. OF REAGENT BLANK EXTRAP. TO OML IS 0 MG OF CARBON
**********STAT

STATION 1 SAMPLER 1 REP 2 CONC = 17.0489
STATION 1 SAMPLER 1 REP 3 CONC = 17.112
STATION 1 SAMPLER 2 REP 1 CONC = 13.4597
STATION 1 SAMPLER 2 REP 2 CONC = 99999
STATION 1 SAMPLER 2 REP 3 CONC = 14.9701
STATION 1 SAMPLER 3 REP 1 CONC = 9.84951
STATION 1 SAMPLER 3 REP 2 CONC = 8.43911
STATION 1 SAMPLER 3 REP 3 CONC = 10.4231
STATION 1 SAMPLER 4 REP 1 CONC = 28.3374
STATION 1 SAMPLER 4 REP 2 CONC = 28.9794
STATION 1 SAMPLER 4 REP 3 CONC = 28.2742
STATION 1 SAMPLER 5 REP 1 CONC = 10.9599
STATION 1 SAMPLER 5 REP 2 CONC = 5.62883
STATION 1 SAMPLER 5 REP 3 CONC = 7.15501
STATION 1 SAMPLER 6 REP 1 CONC = 9.02853
STATION 1 SAMPLER 6 REP 2 CONC = 9.14431
STATION 1 SAMPLER 6 REP 3 CONC = 9.21799
STATION 1 SAMPLER 7 REP 1 CONC = 8.67593
STATION 1 SAMPLER 7 REP 2 CONC = 8.50753
STATION 1 SAMPLER 7 REP 3 CONC = 8.30228
STATION 1 SAMPLER 8 REP 1 CONC = 22.4958
STATION 1 SAMPLER 8 REP 2 CONC = 24.2483
STATION 1 SAMPLER 8 REP 3 CONC = 21.1275
STATION 2 SAMPLER 11 REP 1 CONC = 17.1384
STATION 2 SAMPLER 11 REP 2 CONC = 14.928
STATION 2 SAMPLER 11 REP 3 CONC = 14.9701
STATION 2 SAMPLER 12 REP 1 CONC = 15.6543
STATION 2 SAMPLER 12 REP 2 CONC = 14.3281
STATION 2 SAMPLER 12 REP 3 CONC = 14.6912
STATION 1 SAMPLER 13 REP 1 CONC = 7.11197
STATION 1 SAMPLER 13 REP 2 CONC = 6.91857
STATION 1 SAMPLER 13 REP 3 CONC = 6.69622
STATION 2 SAMPLER 15 REP 1 CONC = 5.00163
STATION 2 SAMPLER 15 REP 2 CONC = 5.01084
STATION 2 SAMPLER 15 REP 3 CONC = 5.13846

****
WEEK 512 RESULTS
****

****
RESULTS FOR STATION 1 SAMPLER 1
MEAN = 18.1663
STANDARD DEVIATION = 1.88105
COEFFICIENT OF VARIATION = 10.3546
****
RESULTS FOR STATION 1 SAMPLER 2
MEAN = 14.2149
STANDARD DEVIATION = 1.06799
COEFFICIENT OF VARIATION = 7.51313
****
RESULTS FOR STATION 1 SAMPLER 3
MEAN = 9.57059
STANDARD DEVIATION = 1.021
COEFFICIENT OF VARIATION = 10.6681
****
RESULTS FOR STATION 1 SAMPLER 4
MEAN = 28.5303
STANDARD DEVIATION = .39
COEFFICIENT OF VARIATION = 1.36696
****
RESULTS FOR STATION 1 SAMPLER 5
MEAN = 7.9146
STANDARD DEVIATION = 2.74552
COEFFICIENT OF VARIATION = 34.6894
****
RESULTS FOR STATION 1 SAMPLER 6
MEAN = 9.13028
STANDARD DEVIATION = .0957231
COEFFICIENT OF VARIATION = 1.04841
****
RESULTS FOR STATION 1 SAMPLER 7
MEAN = 8.49525
STANDARD DEVIATION = .187154
COEFFICIENT OF VARIATION = 2.20304
****
RESULTS FOR STATION 1 SAMPLER 8
MEAN = 22.6238
STANDARD DEVIATION = 1.56426
COEFFICIENT OF VARIATION = 6.9142
****
RESULTS FOR STATION 2 SAMPLER 11
MEAN = 15.6788
STANDARD DEVIATION = 1.26413
COEFFICIENT OF VARIATION = 8.06265

```
**************************************
RESULTS FOR STATION  2  SAMPLER  12
MEAN =  14.8912
STANDARD DEVIATION =  .68521
COEFFICIENT OF VARIATION =  4.60145
**************************************
RESULTS FOR STATION  1  SAMPLER  13
MEAN =  6.90892
STANDARD DEVIATION =  .208061
COEFFICIENT OF VARIATION =  3.01148
**************************************
RESULTS FOR STATION  2  SAMPLER  15
MEAN =  5.05031
STANDARD DEVIATION =  .0765466
COEFFICIENT OF VARIATION =  1.51568
**************************************************************************************

DO YOU WISH TO ENTER THIS DATA IN THE MASTER FILE [Y/N]? Y
 11
 276            20.3381
 11             277             17.0489
 11             278             17.112
 11             363             13.4597
 11             364             99999
 11             365             14.9701
 11             450             9.84951
 11             451             8.43911
 11             452             10.4231
 11             537             28.3374
 11             538             28.9794
 11             539             28.2742
 11             624             10.9599
 11             625             5.62883
 11             626             7.15501
 11             711             9.02853
 11             712             9.14431
 11             713             9.21799
 11             798             8.67593
 11             799             8.50753
 11             800             8.30228
 11             885             22.4958
 11             886             24.2483
 11             887             21.1275
 11             1146            17.1384
 11             1147            14.928
 11             1148            14.9701
 11             1233            15.6543
 11             1234            14.3281
 11             1235            14.6912
 11             1320            7.11197
 11             1321            6.91857
 11             1322            6.69622
 11             1494            5.00163
 11             1495            5.01084
 11             1496            5.13846
```

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