

## Chapter 16. Determination of Total Organic Carbon by a High Temperature Combustion/Direct Injection Technique

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### 1.0 Scope and field of application

This protocol describes a high temperature combustion/direct injection (HTC/DI) technique for the determination of total organic carbon (TOC) in seawater, suitable for the assay of concentrations 30-300  $\mu\text{M C}$ .

### 2.0 Definition

The TOC content of seawater is defined as the total concentration of all non-volatile organic substances expressed as  $\mu\text{moles of C kg}^{-1}$  of seawater.

### 3.0 Principle of analysis

This method of analysis is based upon the complete oxidation of organic compounds to carbon dioxide, followed by the quantitative measurement of this  $\text{CO}_2$  by non-dispersive infra-red (NDIR) analysis. This technique was first attempted for seawater by Sharp (1973) upon modification of a procedure developed by Van Hall *et al.* (1963) for fresh water. Interferences from inorganic carbon in seawater are removed by sparging with  $\text{CO}_2$ -free gas after acidification of the sample (Sharp and Peltzer, 1993).

The instrument response is calibrated by the method of standard additions. Known amounts of organic compounds are added to produce a series of solutions with consistently increasing concentrations of organic carbon. The slope of the regression line obtained when peak area is plotted against the amount of carbon added is the instrument response factor. Glucose dissolved in low carbon water (LCW) is used for this calibration. The principle is the same although the calculations are slightly different. (See section 8.3 below).

The instrument blank is determined by injecting a volume of LCW identical to the volume used during sample analysis (100  $\mu\text{l}$ ) and measuring the peak area. This peak area represents the amount of  $\text{CO}_2$  liberated from the catalyst/combustion tube upon injection

of a liquid sample (the system blank) and so each sample injection must be corrected by subtraction of this amount. It is important that the water used for this purpose be as carbon-free as possible (otherwise over-correction will occur and the TOC concentration will be under-estimated) and that this measurement be repeated throughout the analytical sequence to closely monitor the instrument blank, which may vary over time and use. Until a universally available source of carbon free seawater (CFSW) is developed, low carbon water (LCW) is recommended (see section 7.2).

## 4.0 Apparatus

- 4.1 *Sparging apparatus*: After acidification (50  $\mu$ L 85% phosphoric acid: 50mL sample), samples are sparged to remove > 99.95% of the inorganic carbon. Small volume samples (< 40 mL) can be sparged by bubbling CO<sub>2</sub> free gas (oxygen) through a 1/16 inch Teflon line placed directly in the sample to almost the vessel bottom. A flow-rate of 200 ml min<sup>-1</sup> for a minimum of 10 minutes is usually sufficient to remove all inorganic carbon.
- 4.2 *TOC analyzer*: Several versions of HTC/DI analyzers have been built, either commercially or "homemade". Each of these consists of a furnace and gas processing stream containing the following essential components:
  - 4.2.1 Source of CO<sub>2</sub>-free carrier gas (medical grade oxygen) delivered through a pressure regulator.
  - 4.2.2 High temperature combustion furnace.
  - 4.2.3 Syringe to inject the seawater sample.
  - 4.2.4 Traps to remove HCl, SO<sub>2</sub> and H<sub>2</sub>O.
  - 4.2.5 Aerosol filter.
  - 4.2.6 NDIR CO<sub>2</sub> analyzer.
  - 4.2.7 Peak area integrator

## 5.0 Reagents

### 5.1 Gases

- 5.1.1 *Oxygen*: Medical Grade Oxygen is used for both the sparge and carrier gas. This grade of gas must be cleaned before use, as it contains hydrocarbons. The gas is passed through 3 quartz tubes (19 x 1/4 inch) packed with platinum

coated alumina beads and heated to 720°C in a high temperature combustion furnace. Three tubes ensure that all hydrocarbons present in the oxygen are combusted to carbon dioxide. The carbon dioxide is removed by passing the gas through an Alltech scrubber packed with soda lime. The gas liberated from this clean-up procedure was referenced against Ultra High Purity oxygen and found to be comparable.

## 5.2 *Dry chemicals*

- 5.2.1 *Ascarite*: Thomas Scientific, Swedesboro, NJ.
- 5.2.2 *Magnesium perchlorate* (anhydrous): Aldrich Chemical Co. Milwaukee WI
- 5.2.3 *Soda lime* (+100 mesh): Aldrich Chemical Co.
- 5.2.4 *Cuprox* (copper oxide): Aldrich Chemical Co.
- 5.2.5 *Copper* (10-40 mesh): Aldrich Chemical Co.
- 5.2.6 *Sulphix* (8-20 mesh): Wako Pure Chemical Industries, Ltd.
- 5.2.7 *Platinum Catalyst* (5/64 alumina ball): Shimadzu
- 5.2.8 *Platinum gauze*: Aldrich Chemical Co.

## 5.3 *Solutions*

- 5.3.1 *85% phosphoric acid (concentrated)* (J.T. Baker Inc. Phillipsburg NJ)
- 5.3.2 Glucose stock solution, 10 mM glucose (Sigma) in LCW.
- 5.3.3 *0.1N hydrochloric acid*: prepared by diluting the concentrated acid (J.T. Baker Inc.) in LCW.

## 6.0 **Sampling**

### 6.1 *Sample bottle preparation*

- 6.1.1 40 ml "EPA vials": Vials are soaked in 10% HCl, rinsed three times with Milli-Q water and air-dried. They are then placed in a muffle furnace at 500°C overnight (12-16 hrs), allowed to cool and capped with cleaned open topped green caps lined with Teflon coated septa. The green caps are cleaned by soaking for at least an hour in Milli-Q, rinsing with more Milli-Q and then

allowing to air dry. The Teflon septa are cleaned separately by soaking in 10% HCl and rinsing with Milli-Q three times.

- 6.2 *Niskin bottles*: "well-aged" OTE/Niskin bottles should be used where possible, with silicone O-rings and either epoxy coated stainless steel springs or heavy-walled silicone tubing. The stopcocks may be nylon, polypropylene or Teflon. The bottles should be free of oil and dirt and rinsed thoroughly with fresh water before the ship leaves port. At a test station or at the first station, the bottles should be well rinsed with seawater. Repeated lowerings and firings at 1-2000 m is recommended.
- 6.3 *Drawing of samples*: TOC samples are easily contaminated with organic compounds adsorbed from the air, from fingerprints or from the sampling ports. In order to keep the sampling ports as clean as possible for these samples, no Tygon<sup>®</sup> or phthalate containing tubing should be used in connection with the sampling ports prior to drawing the TOC samples. Gloves are worn during sampling. TOC samples are drawn early, immediately after the gas samples have been drawn. The sample is allowed to flow freely from the OTE bottle for a few seconds to clean the port. The sample bottle is not allowed any contact with the sampling port. The bottles and caps are rinsed three times with a small volume of sample and then the bottle is immediately filled to about 2/3 volume. Filtering does not take place as POC levels are low ( $< 2 \mu\text{mol C kg}^{-1}$ ) and filtering can result in contamination.

#### 6.4 *Sample storage*

- 6.4.1 *Freezing samples*: The samples are placed in an aluminum block (specifically bored-out to maintain a tight fit with the sample vials) cooled to  $-20^{\circ}\text{C}$  to achieve rapid cooling of the samples. After one hour, the samples are checked to see if they are frozen. Super-cooling often occurs. In this case a quick twist of the vial often encourages immediate solidification of the sample with little or no brine formation. Once frozen, samples are moved to a cardboard container for continued storage at  $-20^{\circ}\text{C}$ . Samples are kept frozen until analysis. Thawing and slow re-freezing of the samples is avoided as this encourages fractionation of the samples and brine formation.

## 7.0 **Procedures**

- 7.1 *Column Preparation*: A quartz tube (19 x 1/2 inch) is used as the combustion tube. It is packed starting from the bottom with cuprox (2 g), sulphix (7 g) and platinum catalyst (10 g) to give a head space of 19 cm. Platinum pillows are placed on top of the packing material and act as the catalyst in the combustion of organic carbon. There is a 10 cm holding tube at the bottom of the tube to ensure that the packing material

stays in place. A column should last 10 days in the lab during analysis of about 150 samples.

7.2 *LCW preparation:* Carbon-free distilled water (LCW) can be prepared by a variety of methods. However, no method is refined to the point that it guarantees a low TOC level. Thus it is imperative that the analyst continually checks the quality of the blank water, maintains quality control charts, and crosschecks with other sources and analysts.

7.2.1 *Milli-Q.* The Milli-Q system is capable of achieving quality LCW. This is verified by comparison against LCW used in Sharpe's community wide comparison. Ampules of Sharpe's LCW are archived and referred against the Milli-Q water. When an acceptable level of Milli-Q water is found, a large volume carboy is taken, acidified and ampulated into 5ml ampules.

7.3 *Standard preparation:*

7.3.1 *Distilled water standards:* A series of reference solutions are prepared by sequential addition of the 10 mM glucose standard stock solution to 100 g of distilled water. 25  $\mu\text{M}$ , 50  $\mu\text{M}$ , 75  $\mu\text{M}$  and 100  $\mu\text{M}$  standards are prepared using LCW in 100 mL volumetrics. 100  $\mu\text{L}$  of 85%  $\text{H}_3\text{PO}_4$  is added to each standard and they are sealed and stored at 4°C. The exact concentration of the standards can be calculated directly from the concentration of the stock solution:

$$\text{DOC}(\mu\text{M C}) = \left( \frac{V_{\text{std}} \cdot C_{\text{stock}}}{100 \text{ ml}} \right)$$

Where:

$V_{\text{std}}$  = volume standard

$C_{\text{stock}}$  = concentration of stock solution

7.4 *Blank determinations:* All peak area measurements are corrected for the instrument blank. In order to do this, a LCW sample is injected at regular intervals throughout the day's analysis run (see section 7.5). Typically, six injections of the blank water sample are made in a 10 minute run. This water is acidified and sparged in the same fashion as the samples.

7.5 *Response factor determination:* There are two ways to determine the instrument response factor. The first involves running the complete set of standard solutions.

Generally, this method is used only when a newly packed column has been placed in the furnace. The second involves running only two standards (high and low) spanning the range of concentrations expected for that days run. Typically, this latter method is used on a day to day basis.

- 7.5.1 *Standard addition series:* After running reference waters (deep seawater and LCW) a standard addition curve is run (3 to 5 injections). For a new column a complete set is run (4 point curve) while for a conditioned column a two point curve is run (100  $\mu\text{M}$  and 25  $\mu\text{M}$ ).
- 7.5.2 *CO<sub>2</sub> gas standard calibration:* Both of the proceeding methods assume that complete oxidation of the added standard is occurring. In order to verify this, one can by-pass the uncertainty of the oxidation step by injecting CO<sub>2</sub> in air standards. These should be obtained from a reliable source (e.g. in the U.S., NIST) with the concentration known to a precision of  $\pm < 1$  ppm. The instrument response is calibrated by injecting (in triplicate) a series of volumes. Mean peak areas are plotted versus moles of CO<sub>2</sub> injected, divided by the nominal injection volume. CO<sub>2</sub> is not an ideal gas so the Van der Waals equation of state must be used to calculate the number of moles injected from the observed volume and room temperature and pressure. The slope of this line should be identical with the normal calibration.
- 7.6 *Analytical protocol:* A typical day's run consists of two runs of both deep reference seawater DRSW and the LCW blank, a calibration set, a series of samples run in groups of 4 to 6 with LCW blanks interdispersed, a LCW blank and a DRSW. The LCW and DRSW samples are run to minimize and stabilize the instrument background/blank. The same sample is run repeatedly so it will be possible to see if the instrument blank has stabilized. The sample is run at least 4 times (more if necessary) in order to obtain a repeatable signal prior to beginning the high-low calibration set.
- 7.7 *Sample injection:* Samples are first sparged with CO<sub>2</sub>-free gas (see section 4.1), then the syringe is filled. First, the syringe is rinsed three times with sample, then over-filled and inverted to expel air bubbles. The excess sample is expressed and 100 $\mu\text{L}$  of the sample is then injected into the furnace.
- 7.8 *Post-Analysis:* Following the sample analysis runs, DRSW and LCW blanks must be run, and finally, the LCW used for the day's run is compared with the long-term standard to check for drift and/or contamination. The data are processed according to the equations in Section 8.

## 8.0 Calculation and Expression of Results

- 8.1 *Peak Screening*: Before calculating the mean corrected peak area for each sample, it is imperative that the peak integration be verified. Peak areas may be rejected, (or re-integrated) where an improper baseline or poor or irregular peak shape is observed, or there are other indications of a bad injection. All acceptable peaks for each sample or blank run are averaged.
- 8.2 *Blank Correction*: Early in the lifetime of the combustion tube, the instrument blank tends to slowly decrease. In these cases, the instrument blank between LCW runs is interpolated with a simple linear interpolation to blank correct the sample runs. Later in the combustion tube lifetime, the instrument blank is usually stable. On these days, the instrument blank is averaged over the course of the days run. Mean corrected peak areas are calculated by subtracting the appropriate instrument blank.
- 8.3 *Response factor determination*
- 8.3.1 *Distilled water standard addition series*: The mean corrected peak area is plotted as a function of the concentration of the distilled water standard and a linear regression is fitted to the points. The slope of the line is the instrument response factor in peak area units per micromole.
- 8.4 *Sample analysis*
- 8.4.1 *Blank determination*: The mean peak area for each of the day's LCW runs (in area units) is calculated provided the LCW has remained stable. If the LCW has drifted by 1-2  $\mu\text{M C}$  over the course of the day, the samples are bracketed and the mean peak area is calculated for that set of samples using the run of LCW before the samples and after the samples. As LCW is injected after every 6 samples, this ensures that any drift in the machine system blank is corrected for. Usually a drift only occurs when a new column is involved.
- 8.4.2 *Zero water adjustment*: The LCW used to make instrument blank measurements throughout the day is  $\sim 3 \mu\text{M C}$ . The TOC concentration of the LCW is measured by comparing it to a "primary standard" of TOC free distilled water which has a TOC concentration of  $\sim 3 \mu\text{M C}$  and has been set aside for this purpose.

8.4.3 *TOC calculation:* The following formula is used to calculate the TOC concentration of a sample in  $\mu\text{moles l}^{-1}$  and the concentration of the DRSW:

$$\text{TOC } (\mu\text{MC}) = \frac{S}{R} - B$$

Where:

- S = average sample area; mean peak area (in mV-secs) for four injections of the sample
- R = response factor; instrument slope as appropriate - either the daily mean or the interpolated value (mV-secs  $\mu\text{MC}^{-1}$ )
- B = average system blank, defined as:

$$\text{Blank} = \frac{\text{LCW}}{R} - 3$$

Where:

- LCW = average sample area for LCW
- R = response factor (as defined above)

8.5 The units of  $\mu\text{mole kg}^{-1}$  can be obtained by dividing the calculated TOC concentration by the density of the seawater at the time of analysis.

## 9.0 Quality control/quality assessment

9.1 *QC charts:* In order to have tight quality control over the analyses, the following are plotted on a daily basis. Instrument drift or bad blanks are readily apparent from any trends in the data.

9.1.1 *Daily blanks* (mean +/- standard deviation in  $\mu\text{M C}$  units) are noted in the machine log book. Deep Reference seawater values are also noted and should be in the range of 43-45  $\mu\text{M}$ .



- 9.1.2 *Daily response factors:* The response factor is noted in the machine log book. This rarely changes. The flow into the column and into the LICOR also are monitored daily and noted in the log book, because alterations in flow will change the response factor.
- 9.2 *Quality assurance:* Although the HTC/DI-TOC analytical method has begun to develop some acceptance within the marine chemical community, it is imperative that each investigator demonstrate the validity of their own analyses. This may be accomplished by comparison with DRSW.
- 9.2.1 *DRSW analysis:* In the absence of a CRM (certified reference material) seawater standard, it is possible to simulate one using DRSW. A large volume (>1 l) sample is collected at from >2000 m. The TOC in this sample should be old and relatively stable and recalcitrant. This material is referenced against previously collected water with a TOC concentration of 43-45  $\mu\text{M}$ . Once the reference concentration is met, this material is acidified, ampulated and used as DRSW. This DRSW is analysed several times a day to monitor the performance of the machine. Any significant drift in the concentration of this seawater indicates a problem with the machine.

## 10.0 Notes

- 10.1 *General precautions:* TOC samples are easily contaminated. Stringent anti-contamination protocols must be adhered to at all times. It is important to observe nearby operations which could adversely affect samples.
- 10.1.1 *Sampling:* No post-analysis mathematical correction can salvage poorly drawn or contaminated samples. Every precaution should be taken to collect samples in the cleanest environment possible. TOC samples should be drawn early to avoid contamination from the tubing used as transfer lines in the collection of most samples, Tygon<sup>®</sup> especially. Any sampling prior to the drawing of TOC samples should use Silicone tubing. Above all, fingers (even when gloved) must be kept out of samples.
- 10.1.2 *Sample storage:* TOC samples are also prone to contamination at this stage. Samples should not be stored in refrigerator/freezers which contain organic material or solvents. For BATS, TOC samples are placed in a dedicated TOC contaminant-free freezer.

## 10.2 Possible modifications:

- 10.2.1 *Blank water*: LCW serves as an adequate instrument blank checking material, however carbon-free seawater is unquestionably superior. Development of a process to produce this material quickly, reliably, easily and cheaply is a priority.
- 10.2.2 *Precision*: Historically, TOC concentrations were regarded as both relatively uniform and invariant, in part, due to the relatively poor precision of the analyses. The uncertainties in these older methods were on the order of 10-25% and detailed information was lost to this imprecision. A much more adequate picture of the oceanic carbon cycle is revealed now that precision of  $\pm 1 \mu\text{MC}$  can be obtained. This level of precision ( $\pm 1-2\%$ ) should be achieved by every analyst.
- 10.2.3 *Deepwater reference*: Deep oceanic concentrations of TOC are relatively low and virtually invariant in time. The deep water TOC serves as a natural CRM for controlling the quality of the TOC analyses. Thus where TOC is measured, samples from  $>2-3000$  m should be analyzed as a check of consistency. On the basis of these analyses, results of the newer analytical techniques can be compared to the historical database.

## 11.0 References

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