

PROTOCOLS for Dissolved Organic Carbon Analysis

(I) Introduction and Summary

Organic and Inorganic carbon is found in natural water samples. Total Carbon (TC) is defined as Total Organic Carbon (TOC) plus Total Inorganic Carbon (TIC). TOC forms organic compounds by bonding with hydrogen or oxygen. TIC forms the structural basis for inorganic compounds which includes gas carbonates and carbonate compounds. The concentration of TOC can be derived by subtracting the concentration of DIC from the measurement of TC ($TOC=TC-TIC$). Nitrogen is also readily available in natural water samples and is comprised of organic and inorganic species that are measured together as Total Nitrogen (TN)(Shimadzu 2010).

The TOCL uses High Temperature Combustion Oxidation (HTCO) to measure Non-purgable Organic Carbon (NPOC). In this technique, the TIC component of the sample is volatilized by acidifying the sample to a PH of to 2-3 with subsequent sparging (bubbling) to remove it from the solution. Generally the amount of purgable organic substances in the natural environment is small and NPOC can be considered TOC. After the sample has been sparged it is injected into a combustion tube filled with oxidation catalyst heated to 720C. The sample is burned in the tube and the CO₂ combustion products are moved by a carrier gas through a dehumidifier which cools and dehydrates the sample. It then moves through a halogen scrubber to remove chlorine and other halogens. Finally, the combustion products are introduced to an absorption column coupled to a non-dispersive infrared (NDIR) analyzer. The TOC-L software measures the peak height of the analog signal produced by the detector. The peak height is proportional to the carbon concentration of the sample. Before every run a calibration curve from a standard stock carbon solution is run that mathematically expresses the relationship between peak height and carbon concentration (Shimadzu 2010).

TN may be run simultaneously on the TOC-L. When the sample is introduced to the combustion tube the TN is decomposed to Nitrogen monoxide. The carrier gas introduces the nitrogen monoxide to a chemiluminescence gas analyzer which generates a peak whose area is proportional to TN concentration. A TN calibration curve is also measured before each sample run (Shimadzu 2010).

(II) Sample collection

Avoid contamination, degradation or perturbations

For detailed discussions on sample collection, preservation, and storage, see the following references: JGOFS Report No. 19, 1994; Norrman et al., 1993; Sharp et al., 1993; Tupas et al., 1994

(III) Calibration

Calibration curves are run with a minimum of 4 concentration standards per analysis. For DOC analysis, solutions are prepared from potassium hydrogen phthalate (KHP) with ultra-pure UV-oxidized water ranging in concentration from 40-300 $\mu\text{mol C L}^{-1}$, depending on anticipated range of sample DOC. For TN analysis solutions are prepared from potassium

Nitrate (KNO₃). For samples with broad concentration range, a low and a high standard curve are prepared (e.g., 30-150 and 50-300 µmol L⁻¹).

Standards are weighed and transferred into a class A volumetric flask to make a stock solution. Calibration standards are prepared by using calibrated pipetters to transfer the stock to volumetric flasks.

The calibration standards are transferred from flasks into autosampler vials with open-top teflon-lined septa closures. The TOC-L class of instruments are capable of diluting a working calibration standard (e.g. 300 µmol C L⁻¹ solution) to generate a 4-point calibration curve (75, 100, 150, 300 µmol C L⁻¹). This process typically yields a calibration curve with an R² value >0.999.

The software is set up for the appropriate calibration curves and includes a zero concentration standard (ultra-pure water). Injection volumes of 100 to 150µL are appropriate for the standard sensitivity catalyst. 1.5% HCl addition and a sparge setting of 2 minutes are used. An appropriate number of injections per sample are selected (e.g., 3 of 7 or 3 of 5) that meet the instrument precision criteria such as a standard deviation of ≤0.05 and percent coefficient of variation (%CV) of <2% for 3 of the sample injections (3% for TN).

The calibration curve is set up to account for instrument blank. Instrument blank refers to inherent carbon present within instrument system (primarily the combustion tube/standard sensitivity catalyst assembly) and potentially the ultra-pure water (see Benner and Strom, 1993 and Sharp et al. 2002 for detailed discussions on the topic).

The instrument carbon blank can be determined by routing water from the ultra-pure water trap (when water trap is filled with water), typically port 8 on the 8-port valve to sampling port 1. This water accumulates from the water vapor that condenses upon exiting the combustion tube. Results for the instrument blank can be equivalent to TOC analysis of laboratory ultra-pure water injections if the water purification system is properly maintained.

If the instrument blank response is equivalent to the average response of the ultra-pure water injections average peak area of ultra-pure water blanks is subtracted from the zero concentration standard and all other standards.

A linear regression analysis is performed on the blank-subtracted calibration standards and the regression line is forced through the origin (peak area on the y-axis and DOC concentration on the x-axis). Calibration curves should have an R² >0.999. The slope (response factor) is used to quantify DOC from the peak areas of the seawater samples.

(IV) QC/QA

There is no national or international standard for seawater DOC. However, the scientific community has accepted the deep seawater Consensus Reference Material (CRM) distributed by the Hansell Laboratory, Rosenstiel School of Marine and Atmospheric Science (RSMAS), University of Miami (supported by the U.S. National Science Foundation). The seawater CRM is analyzed at several laboratories that provide consistently high quality results to reach a consensus DOC concentration. Low carbon reference water is also available and shipped with the seawater CRM. Laboratories with high-grade ultra-pure water systems equipped with ultraviolet lamps can produce low carbon water of equivalent quality.

The necks of the CRM ampoules are wiped with dry lint-free tissue or moistened with isopropanol. The ampoules are cracked open and the contents are transferred into clean (pre-combusted) autosampler vials. A clean transfer method is used such as pre-combusted glass Pasteur pipets. The CRM sample is preceded with an ultra-pure water to condition TOC-L system.

The DOC value for the seawater CRM should be within 5% and ideally within 2% of the consensus value. If not, another CRM sample is analyzed and/or acidified ultra-pure water until an acceptable DOC concentration is achieved; otherwise, the entire process is repeated with new KHP stock and calibration standards. The purported precision of the instrument is on the order of 2%.

(V) Formulas used

$DOC = (\text{peak area of sample} - \text{blank peak area}) / C \text{ slope}$

$TN = \text{peak area of sample} / N \text{ slope}$

References:

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