Cariaco Time Series Study

Handbook of Methods for the Analysis of Oceanographic Parameters at the CARIACO Time-series Station

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Prologue

Frank Muller-Karger

The focus of the program "CARIACO Oceanographic Time Series" is the collection of a set of oceanographic, biogeochemical and ecological observations at a specific location (10° 30'N, 64° 40'W), in the deepest area of the Eastern basin of the Cariaco Basin, off the coast of Venezuela. The CARIACO program was established in 1995 with the key objective to contribute critical data for monitoring the carbon cycle and biogeochemistry in the seas of the globe, understand the impact of climate change on biodiversity and regional marine ecology, as also supporting research on past climatic changes. Through the collection of long-term ecosystem observations of the Cariaco Basin with data of very high quality at a reasonable time, the scientific community of Venezuela contributes to the knowledge of mankind in these areas, taking scientific advantage of this particular site.

CARIACO is one of the three longest oceanographic biogeochemical time series stations established around the world. The other two are the Hawaii Ocean Time-series (HOT; 22° 45'N, 158° 00'W) and Bermuda Atlantic Time-series Study (BATS; 31° 45'N, 64° 10'W). HOT and BATS serve as platforms for the study of changes in the Pacific and North Atlantic Ocean, respectively, while the CARIACO time series is located in a tropical environment in a continental margin. The scientific achievements of these three stations have encouraged other countries to develop plans to establish similar time series. The region that examines CARIACO is unique for having an annual primary production which doubles approximately production and vertical flow of organic particulate matter observed in the most oligotrophic waters monitored by HOT and BATS. The CARIACO program also offers the opportunity to study the ecosystem along a gradient oxic/anoxic which lies between 200 and 350 m deep. The Cariaco Basin is also an important place for paleoclimatic studies.

Monthly oceanografic cruises have been conducted in the CARIACO station since October of 1995 to obtain a set of key observations in these oxic and anoxic waters. The objective is to measure the temporal variability and seasonal, interannual and interdecadal trends. The time series is based on the scientific goal of understanding the relationship between hydrography, community and plankton composition, primary production, microbial activity, terrigenous inputs, sediment flows and biogeochemical cycling in the water column, and how changes in these processes are preserved in the sediments.

The CARIACO oceanographic time series is internationally renowned due to numerous factors. Most important one is the understanding that a good observation (a good value) is critical to achieve good science. Scientists, technical staff and students involved in collecting observations within the framework of the CARIACO program are dedicated to continuously monitoring the quality of the data obtained, and review methods and techniques to ensure the highest levels of quality for each data collected. Another essential factor is the close ties established between the scientists involved. Four Venezuelan institutions (Fundación La Salle de Ciencias Naturales-EDIMAR-FLASA, Universidad Simón Bolívar-USB, Universidad de Oriente-UDO, Instituto Venezolano

de Investigaciones Científicas-IVIC) are involved in collecting core observations of the CARIACO program. These institutions have developed programmes of collaboration with numerous international institutions (such as University of South Florida, University of South Carolina, and Stony Brook University). The institutions have close ties that allow frequent communications, fast and effective, information exchange, and technical assistance. Another important factor is in the success to facilitate free access to databases as soon as the quality of the data is acceptable and available. Basic hydrographic, chemical and biological data obtained under the CARIACO project are archived and offered to the public through the Internet portal http://cariaco.ws (USB, Venezuela) who is responsible for developing the permanent database, and in http://imars.usf.edu/cariaco/index.html (University of South Florida)..

The institutions and local authorities support is also key to establish and maintain a program of time series. The CARIACO project benefits from important critical contributions in logistics, infrastructure, interest, and financial support in Venezuela through institutions like the Fondo Nacional de Ciencia Tecnología e Innovación (FONACIT) and FLASA. This last one has served as home base for all field operations and is the center of inter-agency liaison of the project in Venezuela. Administrators, R/V Hermano Ginés crew members and scientists at FLASA have supported and participated in all aspects of the project.

The CARIACO program is also known and respected for its scientific leadership in Central and South America. He has served as model for other national programmes of sea observation in the region. In September 2006, the CARIACO program was recognized at the headquarters of the Venezuela National Navy by the Intergovernmental Oceanographic Commission (IOC-UNESCO/IOCARIBE) for its excellence in research and its contributions to the development of marine sciences in the region.

This manual was written by scientists and technical staff involved in CARIACO, for documenting the methods used in the collection of the set of observations that constitutes the time series program. The manual explains the methods both in Spanish and in English to make them widely available to scientists and technical personnel interested in the CARIACO data and in its operation, and to those who are interested in establishing similar observation programmes. The manual is one of the achievements of the educational effort that takes the project to train scientists and students in data collection of high quality, and the effort that makes this group of people in the dissemination of the generated knowledge. The manual includes the methods used in CARIACO since 1995 with modifications made up to 2010. The inspiration for the development of this publication comes from the methods manual that generated the program US JGOFS BATS (UNESCO, 1994).

Method 1
SAMPLING PROCEDURES

Yrene M. Astor

Introduction

The Cariaco time series station is located at 10°30'N and 64°40'W. The frequency of sampling is monthly and each oceanographic cruise lasts 24 hours. Core measurements require four separate hydrographic casts using a rosette equipped with a CTD, of 12 bottles with a capacity of 8 L. Moreover, radiometric measurements, zooplankton tows and measurements with an Acoustic Doppler Current Profiler (ADCP) are also made. Table 1.1 shows the parameters that are measured in each cruise, and Table 1.2 indicates the sequence of the activities on board during a cruise.

The chief scientist coordinates the logistics of each oceanographic cruise, controls sampling and the successful implementation of the sequence and sampling protocols. Prior to sampling, the chief scientist informs the participants on the cruise of the tasks to perform and reviews with the staff the methods of collecting samples and the appropriate preservation for each parameter. Instructions indicating sampling protocol, preservation methods for each parameter and precautions to follow are placed in visible areas around the rosette and in the laboratories of the boat for possible consultations. The specific protocol of the cruise that indicates the sequence of the casts, parameters to measure and the persons responsible for sampling are also placed in work areas.

Hydrocasts

Measuring and sampling the basic variables at the time series station requires of four hydrocasts: calibration, primary production, inter-deep and shallow. Each cast is done with a 12 bottle rosette system (SeaBird, model SBE 32), and this system is equipped with a CTD (SeaBird, model SBE 25). A summary of the sequence of the hydrocasts, depths to which the bottles are closed, as well as the order in which samples are taken are specified in Table 1.3. The depth of the chlorophyll maximum is determined through the flurometer downcast profile during the primary production cast. The depth of the oxic-anoxic interface is determined with the downcast of the oxygen profile during the intermediate-deep cast (oxygen disappears) and the light attenuation profile (a peak appears when the number of bacteria in the interface increases). Once the CTD-rosette system is on board, it is washed with fresh water and the collection of different samples are carried out immediately.

Table 1.1. Variables measured in the hydrocasts of the CARIACO project.

Parameter	Units	References
Temperature	ºC	SeaBird SBE 3F (6800 m)
Salinity		SeaBird SBE 4C (6800 m)
Pressure	db	SeaBird SBE 29 (2000 m)
Fluorescence	mgChl$a$ m^{-3}	WET labs ECO-FLRTD
Dissolved oxygen	mL L^{-1}	SeaBird SBE 43
Beam attenuation coefficient	m^{-1}	WET Labs C-star
Discrete salinity		Guildline Portasal®
Discrete dissolved oxygen	µM	Aminot and Chaussepied (1993)
pH		Clayton and Byrne (1993)
Hydrogen sulfide	µM	Modify from Cline (1969)
Total alkalinity	µmol kg^{-1}	Breland and Byrne (1993)
Nutrients	µM	Gordon *et al.* (2000)
Particulate organic carbon and nitrogen	µg L^{-1}	Sharp (1974)
Chlorophyll a and phaeopigments	mg m^{-3}	Holm-Hansen *et al.* (1965)
Phytoplankton	Cells mL^{-1}	
Primary production	mgCm^{-3} h^{-1}	UNESCO (1994)
Colored dissolved organic matter coefficient	m^{-1}	Bricaud *et al.* (1981)
Particle absorption coefficient	m^{-1}	Kishino *et al.* (1985)
Total and dissolved organic carbon/Total and dissolved nitrogen	µM	Dickson *et al.* (2007)
HPLC (phytoplankton pigments)		Van Heukelem and Thomas (2001)
Bacteria	Cells mL^{-1}	Hobbie *et al.* (1977)

Table 1.2. Sequence of sampling at the CARIACO station.

Sampling type	Hour	Depth (m)
Calibration cast	4:00	200
Primary production cast	4:30	100
In situ production buoy deployment	~6:00	
Inter-deep cast	7:00	1310
Zooplankton tow	9:00	200
Recovery of production buoy	4 hours after deployment	
Radiometric measurements	11:30	100
ADCP cast	12:30	400
Shallow cast	13:30	160

Tabla 1.3. Sequence of the casts and order of sampling.

DEPTHS (m)	1	7	15	25	mchl*a*	35	55	75	100	130	160	200	250	IN	300	350	400	500	750	1310
4:15 Calibration	Continuous cast without collecting samples																			
4:30 Primary production																				
Primary production	x	x	x	x		x	x	x	x											
Particle absorption coefficient	x	x	x	x	x															
Phytoplankton taxonomy	x	x	x	x	x	x	x	x	x											
Clorophyll and phaeopigments	xx	xx	xx	xx	xx	xx	xx	xx	xx											
HPLC	xx	x	x	x	x	x	x	x	x											
7:00 Intermediate-deep																				
Dissolved oxygen												xx	xx	xx	xx	xx	xx			
pH												xx	x	xx	x	x	x	x	x	xx
Total organic carbon												x		x					x	x
Dissolved organic carbon												x		x						
Particulate organic carbon and nitrogen												x	x	x	x	x	x	x	x	x
Bacteria density												x	x	x	x	x	x	x	x	x
Salinity												x	x	xx	x	x	x	x	x	xx
Total alkalinity												x	x	x	x	x	x	x	x	x
Nutrients												x	x	x	x	x	x	x	x	x
13:30 Shallow																				
Dissolved oxigen	xx	xx	xx	xx		xx	xx	xx	xx	xx	xx									
pH	xx	x	x	x		xx	x	x	x	xx	x									
Colored dissolved organic matter coefficient	x	x	x	x																
Total organic carbon	x					x		x		x										
Dissolved organic carbon	x					x		x		x										
Particulate organic carbon and nitrogen	x	x	x	x		x	x	x	x	x	x									
Bacteria density		x		x		x	x	x	x	x	x									
Salinity	x	x	x	x		x	x	x	x	x	xx									
Total alkalinity	x	x	x	x		x	x	x	x	x	x									
Nutrients	x	x	x	x		x	x	x	x	x	x									
mchl*a* = chlorophyll *a* maximun	x = number of replicates for each depth							IN = interface, límit between the oxic and the anoxic layer												

Method 2
CTD HANDLING AND DATA PROCESSING

Yrene M. Astor and Ramón Varela

Introduction

The following describes the core measurements and data processing activities being undertaken to obtain the CTD continuous profiles of basic parameters such as temperature, pressure and conductivity, and additional parameters such as fluorescence, dissolved oxygen, and light beam attenuation coefficient. The instrument **package** (CTD and rosette) makes oceanographic casts in real time, meaning that the values are read and recorded simultaneously as the cast takes place. The sensors that are part of the package convert environmental data in measurements of voltage or frequency. Based on these variables, salinity, depth, density, etc., can be derived. This chapter describes the type of equipment that is being used at the Cariaco station, its operation and the method employed to process data based on the recommendations of Sea-Bird Electronics (2008). All software is available directly from SeaBird (www.seabird.com).

Equipment

The instrument **package** used at the CARIACO Time Series Station consists of a SeaBird SEALOGGER SBE 25 (instrument for measuring conductivity, temperature and depth), a carousel or rosette (model SBE 32) and a deck unit (model SBE 33). The CTD records at a sampling rate of 8 Hz and has channels for seven auxiliary sensors. The carousel is an electro-mechanical device that triggers the closure of bottles and collection of samples, and is equipped with 12 Niskin bottles. The deck unit supplies energy that controls the carousel and allows the display of data in real time.

Sensors

The CTD includes the following sensors:

Pressure. SeaBird Electronics, model SBE 29; depth range: 2000 m; accuracy: 0.1% of full scale. ***Temperature.*** SeaBird Electronics, model SBE 3F; range from -5 to 35 °C; accuracy: 0.002 °C; resolution: 0.0003 °C; response time: 0.07 s; stability: 0.002 °C yr^{-1}; depth range: 2000 m.

Conductivity. SeaBird Electronics, model SBE 4; range: 0 to 7 S m^{-1}; accuracy: 0.0003 S m^{-1}; resolution: 0.00004 S m^{-1}; response time: 0.06 s with pump; stability: 0.0003 S m^{-1} $month^{-1}$; depth range: 2000 m.

Dissolved oxygen. SeaBird Electronics, model SBE 43; range: 120% saturation level on the surface; accuracy: 2% saturation; stability: 2% in the first 1000 hours with a clean membrane; depth range: 2000 m.

Light transmission. WETlab, model C-Star; path length: 25 cm; response time: 0.167 s; sensitivity: 1.25 mV; wavelength 660 nm; band width: ~ 20 nm; depth range: 6000 m.

Fluorescence. WETlab, model ECO-FLRTD; range: 0.01 to 125 µg L^{-1}; sensitivity: 0.01 µg L^{-1}; linearity: 99% R^2; excitation/emission: 470/695 nm; depth range 6000 m.

Most of these sensors require a constant flow of water to perform the measurements; a ***pump*** (SeaBird Electronics, model 5T, 2000 rpm) controls the flow. All sensors are placed in a cage attached below the

structure of the carousel bottles. Each sensor is connected and set into the cage of the CTD with a specific configuration to obtain precise measurements of water characteristics. SeaBird Electronics provides different settings diagrams for different applications (http://www.seabird.com). The pressure, temperature, conductivity and dissolved oxygen sensors are sent to the manufacturer once a year for maintenance and calibration.

CTD Operation

Once on board and before sailing, test the instrument **package** (CTD and carousel) to verify communication with the CTD, recording of data and bottle triggering. Upon arrival at the station, prepare the CTD for deployment. First, establish communication with the instrument using the program *Seaterm*, verify the status of the instrument, and initialize logging. With the *Seasave* program, prepare the display and format in real time for data storage. All these programs come in the Seasoft package (http://www.seabird.com). Turn on the CTD just before beginning the cast. Lower the instrument **package** to a depth of 7 m and maintain at that level for 5 minutes to equilibrate the sensors. This period of stabilization is necessary so the whole package comes to the surface water temperature, the conductivity cell wets with seawater, the pump is allowed to come on, and sensors and instrument operation can be confirmed (SeaBird Electronics, 2008). Once completed, bring the instrument **package** close to the surface and begin the cast. Before initiating the deployment, stop data acquisition and restart, saving the file with the same name, in order to eliminate the data recorded during the period of stabilization. These data are erratic and inaccurate and are removed during data processing.

Lower the instrument **package** at a speed of 0.75 to 1 m s^{-1} until the desired depth is reached. Verify real-time operation of the sensors with the program Seasave. During the downcast, identify the depths at which water collection is desired. Apart from the pre-established depths for CARIACO (see **Method 1**), other interesting ones are the depth of chlorophyll maximum, depth of the oxic-anoxic interface, etc. Samples are collected during the upcast. The instrument **package** is held for 2 minutes at each depth before closing each bottle to ensure flushing of the bottle and sensor stabilization. The turbulence that is created during the upcast can have an impact on the water collected; therefore, it is advised to wait a reasonable amount of time before closing the bottles. The water conditions at the moment of bottle closure are recorded by the CTD and the information stored in a file with the extension .BL.

The data from each cast is recorded directly into the ship's computer in real time. For each cast, register the file name, time, and GPS position of the ship. Keep a record of the problems that occur while making the cast and any other relevant information about the weather or water conditions.

At the end of each cast, the program Seasave produces three files with the following extensions:

- **HEX.** Representation of the profile information as binary data in hexadecimal format together with a header.
- **CON.** Contains the configuration of the instrument and calibration coefficients used by the program.
- **BL.** Stores the information on water conditions at the time of closing each bottle.

Equipment Maintenance

Before the cruise

1. Check the CTD batteries.
2. The conductivity cell is very sensitive to material deposited in the electrodes and cell. The electrodes are prone to get dirty, and bacterial growth can be another source of impurities. All of this leads to a change in the dimensions of the cell. Wash the cell with a non-ionic detergent (Triton-X) before each cruise.

During the cruise

1. After each cast and when the instrument **package** is on board, turn it off and wash with tap water to remove seawater on electronic instruments, specially rinse the conductivity cell, the bottle triggers and the light transmission and fluorescence meter windows. At the end of the cruise, wash the whole instrument package with soap and water.

After the cruise

1. Lubricate the connections of the instrument with a light layer of silicone grease, which acts as a lubricant and sealant. Avoid lubricants that break the adhesion between nuts and bolts.
2. Check occasionally sacrificial anodes made of zinc.
3. Regularly verify that the hose that connects the water pump with sensors is free of debris, dirt, algae and bacteria growth. This will ensure a smooth flow of water and avoid any contamination of the sensible components of the sensor.
4. Over time, springs and rubber seals on the bottles are weakened, allowing exchange between the sample that is captured and the surrounding water. Examine these parts regularly and repair any leaks or replace any damaged material.

Data Processing

Data processing for each cruise consists of converting the raw data in scientific units, from which variables of interest are calculated, and reduce the size of data matrices to a more manageable scale. Process the data using the software package *SBEDataProcessing-Win32* (SeaBird Electronics, Inc.). The steps to be taken for processing data from the CTD model SEALOGGER SBE 25 are as followed:

Stage 1 processing

1. ***First step.*** Transform the CTD raw data (.HEX file) into scientific units (.CNV file) using the *Data Conversion* module. This module creates a file with the extension .CNV containing the processed data. Graph the data using the module *Seaplot* to check for the presence of values that differ from the average by more than a specific number of standard deviations. Remove outliers using the module *Wildedit* as many times as necessary. In parallel, the *Data Conversion* module creates a file extension .ROS from the file .BL, which contains information on water conditions at the time of bottle closure.
2. ***Determination of advanced coefficients in seconds with respect to pressure***. The CTD sensors are not aligned physically in the instrument **package**. Factors such as the pumping speed, tubing setup, transfer time of water through the tubing, and rocking of the boat generate records that do not correspond to the same parcel of water (SeaBird Electronics, 2008). Use the *Align CTD*

module to correct this. This module aligns the data with respect to pressure and ensures that they characterize the same parcel of water. This module uses different coefficients for each variable, and these coefficients are chosen before starting ***stage 2 of data processing***. For example, the advance of the signal with respect to pressure for the oxygen sensor varies between 3 and 7 seconds. To choose the most adequate time period, run the module Align CTD to the processed CTD file (.CNV file) from the calibration cast using a range of possible advances creating a separate file for each one. Graph the oxygen data for each test using the module Seaplot and choose the value in seconds that minimizes the difference between the downcast and the upcast. The value chosen applies to all files obtained during this cruise when running the module *Align CTD* under ***stage 2 of processing***. This procedure also applies to the light transmission and fluorescence meter data.

Stage 2 processing

1. Once the correct alignment values are established, run the *Filter* module with the processed data (.CNV file). This module 'softens' high frequency data. SeaBird provide the time constants for each variable according to the model of the equipment. For the SBE 25, SeaBird recommends use of a low pass filter with a time constant of 0.5 s for pressure and a time constant of 0.03 s for conductivity.
2. Run the module *Align CTD*. For the SBE 25 with a standard pump of 2000 rpm, SeaBird recommends an advanced conductivity coefficient with respect to temperature of + 0.1 s. The appropriate values for dissolved oxygen sensor, fluorescence and light transmission are determined as described in paragraph 2 of ***Stage 1 processing***. There is no need to align temperature when using the CTD SBE 25.
3. In areas with strong temperature gradients, it is necessary to remove the effects that the conductivity *cell thermal mass* has on the measured conductivity, to minimize the difference between the downcast and the upcast. For that purpose, use the module Cell Thermal Mass. The typical values recommended by SeaBird for thermal anomaly amplitude (alpha) and thermal anomaly time constant (1/beta) for SBE 25 are 0.04 and 8, respectively.
4. Run the *Loop Edit* module to remove the data recorded when the CTD drops/rises below a minimum speed (0.25 m s^{-1}) or when the pressure sensor shows an inverse movement with respect to the direction of the cast due to rocking of the boat.
5. Calculate dissolved oxygen in mL L^{-1} and µmol kg^{-1} using the *Derive* module.
6. Average data with the module *Bin Average* specifying the type of parameter to average (pressure, depth, time or scan number) and the desired range for interval.
7. Calculate parameters such as salinity, sigma-t, potential density, potential temperature, oxygen saturation, etc., using the *Derive* module.
8. Calculate *buoyancy* (Brunt-Väisälä frequency, N) and stability (E) with Buoyancy module.
9. Separate the file into downcast and upcast using the module *Split.*
10. The *Bottle Summary* module reads the file .ROS that is created by *Data Conversion* module and

writes a summary of the data in a file .BTL. This file specifies what the water conditions were at the time of closing the bottles. The file .BTL includes the number and position of the bottle, date/time, and all variables selected.

Stage 3 processing

The following corrections are taken from instructions provided by SeaBird Electronics (2008).

1. ***Correction of salinity with discrete samples.*** During the upcast, take samples for discrete salinity measurements. These are used to calibrate the salinity derived from the CTD conductivity sensor readings. The correction is made as follows:
   a. Examine the processed data obtained during ***Stage 2 processing*** and choose a section of the profile where the values of salinity remain constant.
   b. Within this section, select two depths. From the .BTL files, extract pressure, temperature, conductivity, and salinity of these two depths and put these data into a spreadsheet. Include in a separate column the discrete salinity values for these two depths.
   c. Calculate the conductivity of the discrete sample of the two depths using the module *SeacalcW*.
   d. Calculate the slope between the two depths with the following equation:

$$m = \frac{(A * B) + (C * D)}{(A * A) + (C * C)} \qquad (1)$$

where

A = conductivity of the CTD at depth X.

B = conductivity of the discrete sample at depth X.

C = conductivity of the CTD at depth Y.

D = conductivity of the discrete sample at depth Y.

   e. Introduce this new **slope** value in the conductivity coefficients of a new calibration file (.CON) for the cruise, saving the file with a different name. Repeat ***Stages 1 (First step) and 2*** using the new calibration file.
   f. Verify the validity of the correction, by making a graph of the salinity data of the downcast, before and after the correction, along with the discrete salinities and compare the salinities. If the difference between the corrected data and discrete salinity decreases with respect to the difference between the uncorrected data and the discrete salinity, the correction is valid.

2. ***Correction of dissolved oxygen with discrete samples.*** As with salinity, a series of discrete dissolved oxygen samples are collected only in the oxygenated layer during the upcast and are used to calibrate the data calculated from the records of the oxygen voltage sensor. The correction is made as follows:

   a. From the .BTL files, extract pressure, depth, temperature, SBE 43 voltage, and oxygen saturation in mL L^{-1} of the depths where discrete samples were collected in the casts. Introduce these data into a spreadsheet. Include in a separate column the discrete dissolved oxygen values obtained with Winkler titration.
   b. Calculate **TcorT** with the following formula:

$$\mathbf{TcorT} = Tcor * T \qquad (2)$$

where

Tcor = Tcor coefficient found in the calibration file.

T = water temperature (°C) for each depth.

c. Raise the estimated value **TcorT** to the exponential ($\mathbf{e}^{TcorT}$).

d. Calculate **PcorP** with the following formula:

$$\mathbf{PcorP} = Pcor * P \quad (3)$$

where

Pcor = Pcor coefficient found in the calibration file.

P = pressure (db).

e. Raise the estimated value **PcorP** to the exponential ($\mathbf{e}^{PcorP}$).

f. Calculate Phi with the following formula:

$$Phi = e^{TcorT} * OxiSat(T,S) * e^{PcorP} \quad (4)$$

where

OxiSat (T,S)= saturation level (in mL L^{-1}) to the temperature and salinity at which the sample was captured.

g. Make a graph O_2Winkler/Phi versus voltage of SBE 43 sensor.

h. Calculate the linear regression of the data.

i. The calibration coefficient **Soc** for the oxygen sensor is equal to the slope of the regression line, and the **Voffset** ratio is equal to the constant of the linear regression divided by the slope.

j. Introduce these two new factors in the oxygen coefficients of a new calibration file (.CON) for the cruise and repeat ***Stages 1*** (***First step***) ***and 2*** using the new calibration file.

k. Verify the validity of the correction, establishing differences between both the CTD uncorrected dissolved oxygen values and the corrected ones with discrete dissolved oxygen values obtained with the Winkler titration.

l. Make a graph of these differences against discrete values. Examine whether the correction reduces the difference between the CTD data and the discrete values.

m. Make a graph of the CTD uncorrected dissolved oxygen data along with the corrected ones and the discrete samples. Examine whether the corrected data agrees with the discrete samples.

3. ***Correction of light transmission data.*** This sensor measures both light transmission (%T) and beam attenuation coefficient (c). Calibration should be carried out in air shortly before the cruise, preferably in a laboratory under stable conditions. Once the calibration is completed, calculate the new coefficients and change them in the calibration file (.CON). This allows having a real-time value adjusted for the sensor that does not require a later correction.

   For calibration, follow the instructions provided by SeaBird in its Application Note No. 7, March 2004. The values are:

$$\text{Light transmission (\%)} = (M * \text{output voltage}) + B \quad (5)$$

$$\text{Attenuation coefficient (c)} = (1/z)*\ln(T) \quad (6)$$

where

M, B= calibration coefficients.

z = path length of light beam in meters (0.25 m Wet Labs model C-Star).

T = light transmission (in decimals) which is the transmission in percentage divided by 100.

a. ***Calculation of M and B.*** Connect the CTD to a computer and with the *Seaterm* program choose the configuration option SBE 25. Without turning on the CTD, activate commands >**ds** to awaken the CTD and >**vr** to turn on the sensors. The display generates a list of voltages of each sensor separated by columns. Block the path of light with a piece of black felt to find out which column corresponds to the light transmission sensor. The voltage values that tend towards zero are those belonging to this sensor. Clean light transmission sensor windows to eliminate any stain or coating with lens paper moistened with distilled water, free from any impurity or dust, and dry with a new lens paper. Do not rub strongly on the windows. Repeat this operation about three times or until the voltage on the screen reaches a maximum value. Write down this voltage as "A1". Proceed to block the light beam with a black felt on the side window of the sensor. Write down the voltage measured as "Y1".

The calculations to determine M and B as indicated by SeaBird are:

$$M = \left\{\frac{(T_w)}{(W0 - Y0)}\right\} * \left\{\frac{(A0 - Y0)}{(A1 - Y1)}\right\} \quad (7)$$

$$B = -M * Y1 \quad (8)$$

where

A0= output voltage in air, supplied in the calibration certificate

Y0= output voltage in darkness or zero (light path blocked) provided in the calibration certificate.

W0= output voltage in pure water supplied in the calibration certificate.

T_w= percent transmission in pure water, relative to water (100%, the estimated value provides attenuation coefficient, c_p, due to particles) or to air (90.2% using this percentage the attenuation coefficient calculated is "c" which is equivalent to the attenuation due to water and particles).

A1= voltage measured when adjusting the calibration in air.

Y1= voltage measured when adjusting the calibration during the light path blocking.

Change the coefficients M and B in the calibration file (.CON) using the *Seasave* program.

NOTE. In CARIACO, the value estimated is the beam attenuation coefficient caused by particles or water, c_p (660), T_w = 100. For the beam attenuation coefficient caused by particles and water "c", the constant c_w = 0.364 m^{-1} is added to c_p (Bishop, 1986).

This adjustment is performed to correct the drift of the equipment. If the amplitude of the adjustment is too big, it indicates a strong drift or appropriate adjustment is not reached after several attempts. Send the equipment to the manufacturer for a checkout and calibration. Verify the equipment performance during its operation when measuring the transmission of light in deep ocean waters (> 1000 m) virtually free of particles.

In this case, the light attenuation coefficient "c" should be close to pure water c_w = 0.364 m^{-1}. At the CARIACO station, the particle minimum is found at 500 m deep.

4. ***Correction of fluorometer data.*** The goal to measure the *in situ* fluorescence **(F)** is to determine the distribution of phytoplankton in the water column. The level of **F** varies considerably in direct relation to the amount of chlorophyll present in response to environmental conditions, taxonomic composition and cells physiological conditions. **F** is expressed in output voltage units of the equipment or in relative units. Only when there is a calibration between in situ chlorophyll (Chl*a*) samples and measurements of **F** it is possible to report corrected F in chlorophyll units of mg m^{-3} or µg L^{-1}. It is important to perform this calibration with samples collected at different depths as the relationship between Chl*a* and **F** varies along the water column (Rueda, 2000). Note that the data generated by the fluorometer during CTD processing are expressed in units equivalent to mg m^{-3} of chlorophyll because this is specified in the CTD configuration file. This does not mean this is correct.

Adjustment

The adjustment is derived from the expression:

$$\text{Chl}a = \frac{F}{(m * z + b)} \qquad (9)$$

where

Chl*a* = chlorophyll concentration estimated from the fluorescence.

F = *in situ* fluorescence equivalent to mg m^{-3} of chlorophyll.

z = depth (m).

m = slope of the linear regression line.

b = linear regression constant.

The steps to follow are:

a. Extract the value of **F** from the .BTL file at the same depths at which discrete Chl*a*, samples were taken (eight depths in the case of CARIACO).

b. Calculate the relationship **F**/Chl*a* for each discrete depth.

c. Make a graph with the value of the relationship **F**/Chl*a* versus depth. Calculate the correlation and linear regression (m and b) of this set of data. The correlation can be strong or weak, as well as the sign of the slope can be positive or negative.

d. Using a spreadsheet, copy the columns with the values of depth and the value of **F** from the .CNV file. Calculate the corrected **F** ($\mathbf{F}_{corr}$) in a third column.

$$F_{corr} = \frac{F}{(m * z + b)} \qquad (10)$$

e. Make a graph with the **F** uncorrected data, along with $\mathbf{F}_{corr}$ and discrete samples. Examine whether the corrected data coincide with the discrete samples. In most cases, the adjustment is acceptable.

Method 3
DETERMINATION OF SALINITY

Yrene M. Astor

Introduction

This procedure describes the method for the determination of salinity in seawater within the range of 0.004 to 76 mS cm^{-1} (salinity 2-42).

Principle of Analysis

The Practical Salinity Scale 1978 (PSS 78) defines the salinity of seawater as the conductivity ratio (K_{15}) between a seawater sample at a temperature of 15 °C and pressure of 1 atm to that of a KCl solution with a mass fraction of 32.4356 g kg^{-1} of solution at the same temperature and pressure (Lewis and Perkins, 1981). A salinometer is used to measure this conductivity relationship at a constant temperature. This instrument compares constantly the seawater sample conductance against an internal reference, which is standardized against the IAPSO standard seawater.

Analytical Warnings

1. Conductivity is a measurement of the mobility of the ions to carry a charge through a solution. Since conductivity is affected by temperature, all measurements should be made at a constant temperature. When temperature rises, the thermal movement of ions increases; therefore conductivity increases.

Materials

1. Transparent glass bottles, 250 mL capacity, with plastic screw cap and liner. Bottles are kept with water from previous sampling to prevent the salt crystal buildup due to evaporation and to maintain equilibrium with the glass (Knap *et al.*, 1997). Every six months, acid wash the bottles (10% HCl) and rinse with distilled water.

Equipment

1. Salinometer Guildline, Portasal® model 8410A. Range: 0.004 to 76 mS cm^{-1} with a resolution of ± 0.0003 mS cm^{-1} (S = 35 and 15 °C). Accuracy ± 0.003 units of practical salinity.

Reagents

1. ***Standard Seawater IAPSO*** for calibration of the instrument.

Sampling

1. Discard the water remaining in the bottle and rinse three times with the new sample. Fill up to the bottleneck, and dry the mouth and the lid with a paper towel before closing. Collect a sample with a replicate for each cast.
2. Keep the bottles at room temperature away from the light and in an upright position. Sample analysis must be made no later than one month after the cruise.

Sample Analysis

1. The salinometer is calibrated according to manufacturer's recommendations (Guildline Instruments, 2006) using standard seawater IAPSO. A standard solution is used to calibrate the equipment at the beginning of the analysis, and a second calibration is done at the end to check the drift of the apparatus.
2. Samples are open only at the time of analysis.
3. Shake the bottle gently to eliminate any gradient present in the bottle before opening. Three measurements are made for each sample.

Calculation and Expression of Results

The salinity calculations are based on the definition of Practical Salinity Scale (Lewis and Perkins, 1981; Unesco, 1981).

Quality Control

A graph of the results is made against depth and values are compared with average salinities ± 2 σ for each depth calculated from the discrete samples of the Time Series data. In addition, the data is matched with the salinity CTD data to identify outliers. Duplicate samples from deep water (Z = 1310 m) agree ± 0.003 units of salinity from cruise to cruise.

Method 4
DETERMINATION OF HYDROGEN SULFIDE

Xiaona Li and Yrene M. Astor

Introduction

This procedure describes the method for total dissolved sulfide (H_2S, HS^- and S^{2-}) in seawater using a spectrophotometric method described by Cline (1969) and modified according to Hayes *et al.* (2006). The results are expressed in units of µmol L^{-1} of sulfide in seawater. The range of concentration is between 0 and 1000 µmol L^{-1}. The accuracy of the method is 3.3% and the detection limit of the analysis is 0.6 µmol L^{-1}.

Principles of Analysis

Hydrogen sulfide present in seawater samples reacts with a solution of acidic ferric chloride and N,N-dimethyl-p-phenylenediamine sulfate to form methylene blue which can be subsequently measured by spectrophotometry.

Analytical Warnings

1. The presence of bubbles, agitation and sample manipulation during sample collection or handling of standards can result in errors because they can introduce oxygen to the sample.
2. The stock and standard hydrogen sulfide solutions are very unstable and the concentration of the standard solution can change quickly (before the $Zn(Ac)_2$ fixing reagent is added). It is important to perform the analysis in less than 2 hours after addition of the diamine.
3. Color development depends on the pH of the sample; therefore, dilutions are made with seawater free of sulfur to buffer pH.
4. The presence of thiosulfate in the sample may inhibit the reaction that develops color, the inhibition time depending on the concentration of thiosulfate.

Materials

1. Automatic pipette with capacity of 5 mL.
2. Vials with a capacity of 20 mL.
3. Glass syringe, capacity 10 mL (Hamilton gas-tight) with Teflon® tip plunger. This syringe should be tested to make sure bubbles could easily be expelled from syringe. Some syringes have a shape that makes this very difficult, so several syringes should be purchased and tested prior sampling.
4. Plastic syringe, capacity 60 mL, with Tygon® tubing 60 cm in length, connected to its tip.
5. Transparent Tygon® tubing for water sampling from the Niskin bottle, internal diameter no greater than 4 mm. Use silicon Tygon® tubing in case dissolved or total organic carbon samples are taken afterwards. Soak the tubing in clean seawater for 24 hours before the cruise to minimize the amount of bubbles that stick to the tubing during sampling.

Equipment

1. Spectrophotometer, equipped with cells with optical pathlength 1 and 10-cm.

Reagents

Depending on the concentration of sulfur present in the sample, the concentrations of reagents and dilution factors vary. See Cline (1969) for samples with concentrations lower than 3 or more than 40 µmol L^{-1}.

1. ***Zinc acetate solution (0.05 M).*** Weigh 1.1 g of zinc acetate ($Zn(CH_3COO)_2$, $Zn(Ac)_2$ and dissolve in 100 mL of distilled water. The reagent is stable and it is used to fix the sulfide.
2. ***Mixed diamine solution (sulfide concentrations between 0 and 44 µmol L^{-1}).*** Weigh 4 g of analytical reagent grade ferric chloride ($FeCl_3$) and 1.6 g of N,N-dimethyl-p-phenylenediamine sulfate ($C_8H_{12}N_{12}H_2O_4S$, molecular weight: 234.28 g mol^{-1}). Add to 100 mL 6N HCl and mix. The reagent is stable for a month if stored in an amber bottle at 4 °C.
3. ***High purity Nitrogen gas.***
4. ***Distilled water free of oxygen.*** Prepare boiled distilled water and let it reach room temperature while bubbling with nitrogen gas.
5. ***Stock sulfide solution.*** *Prepare just before beginning the analysis.* Pour aprox. 230 mL degassed distilled water while the water is still hot (to minimize oxygen diffusion) into a 250 mL volumetric flask. Bubble nitrogen gas into the flask while the water reaches room temperature. Blot ca. 0.5 g of analytical quality sodium sulfide ($Na_2S.9H_2O$) with a paper towel that does not release fibers. Write down the exact weight of the solid to calculate the exact concentration of the stock and standard solution. Add the solid to the 250 mL flask, mix and fill to the line of the volumetric flask with degassed distilled water at room temperature. Dissolve the solid completely. .
6. ***Standard sulfide solution.*** Pour 90 mL of degassed distilled water, still hot, into a second 100 mL volumetric flask, bubble nitrogen gas into the flask while the water reaches room temperature. Pipet 5 mL stock solution to make a more diluted standard solution. This solution should be used immediately.

   NOTE. During the preparation of the stock solution, keep air contact to a minimum; hence, both the weighing of the reagent as well as the dissolution in the flask should be implemented as quickly as possible. This solution as well as the standard solutions are very unstable; therefore, the stock solution preparation and calibration curve should be made as quickly as possible.
7. ***Sulfuric acid solution*** (1:1, v/v).
8. ***Starch indicator.*** Dissolve 1 g of soluble starch in 100 mL of distilled water and heat until turbidity disappears. Add a few drops of chloroform as preservative and refrigerate. This solution is stable for 2 weeks. Renew again if a pure blue color is not developed when titrating..
9. ***Standard thiosulphate solution (0.01 N).*** Dissolve 2.48 g of sodium thiosulphate ($Na_2S_2O_3.5H_2O$) in 1000 mL of distilled water. This solution is unstable, so prepare before each analysis and keep refrigerated in an amber bottle if not used immediately. Normalize every day.
10. ***Potassium iodate standard solution (0.01 N).*** Dry the potassium iodate (KIO_3) in an oven to 105 ºC for one hour, cool in a desiccator and weigh exactly 0.3567 g. Dissolve in distilled water and adjust the volume to 1000 mL. This solution is stable indefinitely if evaporation is prevented.
11. ***Potassium iodide solution.*** Dissolve 20 g of potassium iodide (KI) in 100 mL of distilled water.

Sampling

Samples are taken in triplicate and should be the first ones to be sampled from the Niskin bottle. Hydrogen sulfide is volatile, and it oxidizes very rapidly when exposed to oxygen; therefore, it is important to take precautions during sampling to prevent this interference.

1. Place a 60 mL plastic syringe barrel in a ring stand clamp and connect the Tygon® tubing to the spigot of the Niskin bottle (Figure 4.1). Allow the flow of water to fill the barrel from the bottom, rising up through the syringe. Flow should be continued at a slow rate during sampling, which minimizes contact with air and in turn prevents the formation of bubbles. Place the tip of the gas-tight syringe below the surface of the ascending flow and rinse the glass syringe three times with the water sample. Vent any bubbles that are trapped after the first rinse. Collect 4 mL of sample while maintaining the time of contact between the piston and the sample to a minimum. Avoid the formation of bubbles during sampling.
2. Place the sample in a 20 mL vial slightly tilted that contains 0.5 mL $Zn(Ac)_2$ as fixing reagent. The inclination reduces the reaction time between the sulfur in the sample and $Zn(Ac)_2$. Screw top on tightly and stir gently but make sure that any $Zn(Ac)_2$ on cap is included.
3. Store the sample at 4 °C in the dark as soon as possible. At this temperature, the sample can remain stored for up to one month.
4. In a plastic bottle, collect enough water from a depth with a similar alkalinity to the sample so buffering will be similar between samples and standards. Filter and store at 4 °C. This water **free of sulfur** is oxygenated and used for pH control for the various dilutions to be done both to samples as well as to the standard solutions.

Sample Analysis

Turn on the spectrophotometer 15 minutes before beginning the analysis to stabilize.

1. Samples must reach room temperature before beginning analysis.
2. Add 0.05 mL (50 µL) of the mixed diamine solution with an automatic pipette. Stopper immediately and shake the vial.
3. Let the solution stand for 20 minutes protected from light.
4. Dilute sample with 5 mL of seawater free of sulfide. Cover and mix quickly. Let the solution stand again for 15 minutes protected from light.
5. Measure the absorption of the samples in a 1-cm cell at 670 nm for samples containing sulfur concentrations greater than 3 µmol L^{-1}. For those with concentrations lower than 3 µmol L^{-1} use a 10-cm cell.
6. Samples with high concentrations of hydrogen sulfide may have to be diluted once the color has fully developed. However, this is not recommended as it increases the uncertainty of the method. The highest absorption should be less than 0.8 since the aqueous methylene blue solutions do not conform to Beer's law at high concentrations (Cline, 1969).

Calibration

1. The range of concentration of the calibration curve should cover the range expected in the samples to study. See Cline (1969) for more details about reagents preparation and dilution factors for concentrations greater than 40 µmol L^{-1}.
2. ***Solutions for standard curve.*** For a sulfur concentration range between 0 to 40 µmol L^{-1}, volumes

of 0.1, 0.2, 0.3 and 0.4 mL of the standard solution are added in vials containing 0.5 mL of $Zn(Ac)_2$.

3. Dilute with 4 mL of seawater free of sulfide, and add 0.05 mL (50 µL) of the mixed diamine solution with an automatic pipette. Cover and invert the vials.
4. Allow the diluted standards to rest for 20 minutes protected from light.
5. Dilute with another 5 mL of seawater free of sulfide, and let stand again for 15 minutes protected from light.
6. Measure the absorption of the standard solutions in a 1-cm cell at 670 nm. The concentration of sulfur in each vial is calculated from the amount of sodium sulfide added and dilution factors.

Standard Solution Calibration

Carry out the calibration of the standard solution immediately after its preparation:

1. Add 10 mL of potassium iodide solution and 10 mL of the potassium iodate solution to six Erlenmeyer flasks with barrelhead stopper.
2. Add 1 mL of H_2SO_4 to each flask.
3. With a calibrated volumetric pipette, add 50 mL of the sulfide standard solution to three of the flasks, and 50 mL of oxygen-free distilled water to the other three flasks.
4. Allow to stand for 10 min.
5. Titrate with standard thiosulfate solution using a starch solution as indicator.
6. The volume of thiosulfate among replicates should agree to within ± 0.05 mL.
7. Calculate the amount of H_2S present with the following formula:

$$\mu\text{mol L}^{-1}\ H_2S = 10^6 * M * \frac{(A - B)}{2V} \qquad (1)$$

where

A= average titers of the three solutions without sulfur, in mL.

B= average titers of the three solutions with sulfur, in mL.

V= volume of the sulfur standard solution, 50 mL.

M= concentration of the thiosulfate solution (mol L^{-1}).

Blank Analysis

The amount of hydrogen sulfide in seawater with oxygen is negligible, so it can be used as a blank.

1. Place in a vial 0.5 mL $Zn(Ac)_2$ and add 4 mL of seawater free of sulfide.
2. Add 0.05 mL of the mixed diamine solution with an automatic pipette, stopper and mix.
3. Let the solution stand for 20 minutes.
4. Measure the absorption in a 1-cm cell at 670 nm. The value obtained depends on the purity and concentration of the reagent, turbidity of the sample and the difference between cells (Cline, 1969).

Calculation and Expression of Results

The amount of hydrogen sulfide dissolved in seawater is calculated using the following equation:

$$H_2S(\mu\text{mol L}^{-1}) = \frac{(2.388 * (Abs_s - Abs_b))}{p} \qquad (2)$$

where

2.388 = dilution factor (calculated from the volumes of $Zn(Ac)_2$ + sample + water free of sulfur + diamine solution)/4.

Abs_s = absorption of the sample.

Abs_b = absorption of blank.

p = slope of the calibration linear curve calculated from sub-standard solutions with absorption less than 0.8.

Method 5
DETERMINATION OF DISSOLVED OXYGEN

Yrene M. Astor

Introduction

This procedure describes the method for the determination of dissolved oxygen in seawater through a technique based on the Winkler method (Winkler, 1888; Carpenter, 1965), modified by Aminot and Chaussepied (1983). The detection limit is 0.06 mL L^{-1}, the accuracy of the method is ± 0.03 mL L^{-1}.

Principle of Analysis

Dissolved oxygen in the sample reacts with manganese in a strong alkaline medium to generate a precipitate ($MnO(OH)_2$). The sample is then acidified to a pH between 1.0 and 2.5 that would dissolve the precipitate and release iodine (added with the alkaline solution) in quantities equivalent to chemically dissolved oxygen content in the original sample. The iodine released is titrated using a standardized solution of sodium thiosulphate, which reduces the iodine to iodide. An indicator solution (starch) detects the endpoint of the titration. With this method, four moles of thiosulphate are equivalent to one mole of dissolved oxygen (Aminot and Chaussepied, 1983).

Analytical Warnings

1. In waters with high concentrations of oxidizing agents and/or reductants (iron, sulfite, thiosulphate, nitrite, etc.), positive or negative interferences are present. Organic compounds susceptible to fix iodine as well as substances that are oxidized in an acid medium are another source of error (Aminot and Chaussepied, 1983).
2. Introduction of air bubbles inside the bottle as well as in the Tygon® tubing used for sampling produces positive interference.
3. The transfer of the sample as well as vigorous agitation favors the dissolution of air in the sample and the loss of iodine because of its volatility. This is avoided titrating in the same flask where the sample is taken.
4. The titration should be done quickly to avoid atmospheric oxidation of iodide to iodine.
5. The visualization of the titration endpoint is difficult in waters that have elevated levels of color due to the presence of organic matter as well as of turbidity.

Materials

1. Volumetric pipettes (10 and 100 mL).
2. Automatic dispensers (0 to 2 mL).
3. Automatic pipettes with capacity of 1 mL.
4. Pyrex flasks, 125 mL capacity, used for determining iodine, with barrelhead stopper that project above liquid seal trough to facilitate removal of stopper. Wash the flask previously with distilled water and dried completely. Each flask and stopper must be identified and kept together at all times. The volume of the flask with the stopper on should be calculated gravimetrically (see section “Determination of Sample Volume”).
5. Tygon® tubing for sampling the Niskin bottle, with a

diameter no larger than 4 mm. Use silicon tubing of the same diameter in case total or dissolved organic carbon (TOC and/or DOC) are taken following oxygen sampling.

Equipment

1. Piston burette with a resolution of 0.01 mL.
2. Magnetic stirrer.
3. Analytical balance, with appreciation of 0.1 mg.

Reagents

1. ***Manganese solution***. Dissolve 600 g of manganous chloride tetrahydrate ($MnCl_2.4H_2O$), or 670 g of manganous sulphate tetrahydrate ($MnSO_4.4H_2O$), or 560 g of manganous sulphate dihydrate ($MnSO_4.2H_2O$), or 510 g of manganous sulphate monohydrate ($MnSO_4.H_2O$) in 1000 mL of distilled water. Keep at room temperature.
2. ***Alkaline iodide solution.*** Dissolve 320 g of sodium hydroxide (NaOH) in 300 mL of distilled water. Dissolve 600 g of sodium iodide (NaI) in 300 mL of distilled water while the solution is warmed up. Mix the two solutions at room temperature and adjust the volume to 1000 mL. If the samples have nitrite in quantities > 3 µmol L^{-1}, add 10 g of sodium azide (NaN_3) dissolved in 40 mL of distilled water before adjusting the volume. Keep at room temperature.
3. ***Sulphuric acid solution.*** Dilute 280 mL of concentrated sulphuric acid (H_2SO_4, d = 1.84 g mL^{-1}) in distilled water. Adjust the volume to 1000 mL.
4. ***Starch indicator.*** Dissolve 1 g of soluble starch in 100 mL of distilled water and heat until turbidity disappears. Add a few drops of chloroform as preservative and refrigerate. This solution is stable for 2 weeks. Renew again if a pure blue colour is not developed when titrating.
5. ***Standard thiosulphate solution (0.01 N).*** Dissolve 2.48 g of sodium thiosulphate ($Na_2S_2O_3.5H_2O$) in 1000 mL of distilled water. This solution is unstable, so prepare before each analysis and keep refrigerated in an amber bottle if not used immediately. Normalize every day.
6. ***Potassium iodate standard solution (0.01 N).*** Dry the potassium iodate (KIO_3) in an oven to 105 ºC for one hour, cool in a desiccator and weigh exactly 0.3567 g. Dissolve in distilled water and adjust the volume to 1000 mL. This solution is stable indefinitely if evaporation is prevented.

Determination of Sample Volume

The volume of sample in each flask should be known with precision. The volumes are obtained by weight as follows:

1. Mark each flask along with their stoppers.
2. Wash and rinse with distilled water each flask, dry in an oven approximately at 100 °C and allow it to reach room temperature.
3. Weigh each empty flask with its stoppers.
4. Fill the flask with distilled water and put the stopper on without leaving any air bubbles inside. Dry thoroughly, especially around the cap and at the edges with absorbent paper.
5. Weigh the flasks with distilled water.
6. Measure the temperature of distilled water inside the flasks.
7. Calculate the volume of water with the following formula:

$$V_{(mL)} = \frac{(P_2 - P_1)}{D} \quad (1)$$

where

P_1 = weight of the empty flask (g).
P_2 = weight of the flask with distilled water (g).
D = density of distilled water at the temperature of the measurement (g mL^{-1}).

The density of distilled water at different temperatures is shown in Table 5.1.

Sampling

1. The samples for dissolved oxygen are collected from the Niskin bottles after taking samples for hydrogen sulfide. Samples are taken with a replicate.
2. Transparent flexible Tygon® tubing is used to collect the sample. This reduces the introduction of air bubbles while the sample is taken. Raise the end of the hose, open the water flow and remove air bubbles by pressing the tubing with your fingers. Place the flask in an inverted position and locate the tubing to the bottom of it. Rinse the flask and its stopper twice with the sample before sampling. Place the flask in upright position and allow the water overflow before stoppering. Calculate the volume of water that should overflow by estimating the time the bottle takes to be filled in and then allow water to continue overflowing for a period equivalent to twice that time. While filling the flask hit the bottom with the stopper to prevent any bubbles to adhere to the glass. Remove the hose, by slowly lower the flask until the hose is ~ 1 cm below the water surface. Close the flow and remove the tubing with care.
3. Before placing the stopper, add[1] immediately 0.8 mL of the manganous solution and 0.8 mL of the alkaline solution, placing the tip of each dispenser below the surface of the sample while trying to avoid the formation of bubbles while adding the reagents. Stopper firmly, without remaining bubbles inside, and mix by inversion at least 15 times. Allow the precipitate to settle half way down the flask about 20 minutes and mix again.
4. Place seawater into the barrelhead stopper to avoid dryness and gas diffusion. Keep the flask at room temperature protected from light.
5. Analyze samples over a period no longer than 24 hours after sampling.

Sample Analysis

1. Before the analysis, allow the samples and reagents reach room temperature.
2. In the laboratory, withdraw the water that is around the barrelhead stopper top of the flask before beginning the analysis, trying not to cause turbulence in the sample that could disturb the precipitate. Remove the stopper and carefully add 0.8 mL of sulphuric acid solution. Mix the solution with a magnetic stirrer to dissolve the precipitate completely.
3. Titrate directly into the flask in less than an hour with sodium thiosulfate solution 0.01 N until obtaining a pale yellow colour.
4. Add 1 mL of starch indicator, which turns the solution blue. Continue titrating slowly until the solution becomes transparent and remain so for 20 seconds. The titration should not take more than 3 minutes.

[1] The dose of each reagent depends on the flask volume used at a proportion of 6 mL per liter of sample.

Thiosulphate Calibration

A minimum of three standards for each group of samples should be done.

1. Add 10 mL of the potassium iodate standard solution 0.01 N in a clean flask.
2. Add approximately 100 mL of distilled water.
3. Add 0.8 mL of sulphuric acid solution and mix.
4. Add 0.8 mL of the alkaline solution and mix.
5. Titrate immediately following steps 3 and 4 of "Sample Analysis".

Determination of Blank

Analysis of blank (B_{ana}). Reagents may contain reducing or oxidants impurities that may be controlled by doing a blank.

1. Add 1 mL exactly of the potassium iodate standard solution 0.01 N to a very clean flask.
2. Pour 100 mL of distilled water with a volumetric pipette.
3. Add 0.8 mL of sulphuric acid solution and mix.
4. Add 0.8 mL of the alkaline solution and mix.
5. Finally add 0.8 mL of the manganous solution and mix.
6. Titrate immediately until the point of equivalence (V_1).
7. Add again 1 mL of the potassium iodate 0.01 N standard solution and titrate immediately with thiosulphate (V_2).
8. The analysis blank is calculated with the formula:

$$B_{ana} = (V_1 - V_2) \quad (2)$$

Standard blank (B_{std}). Repeat steps 1 to 8 as described for Bana but do not add manganous solution.

Calculation and Expression of Results

The concentration of dissolved oxygen is calculated using the following equation:

$$O_2\ (mL\ L^{-1}) = 559.8 * \frac{(VT - B_{ana})}{(V_{std} - B_{std})} * (V_{sam} - V_r) - 0.01 \quad (3)$$

where

VT= thiosulphate volume used for titrating the sample.

B_{ana}= analysis blank.

V_{std}= average volume of thiosulphate used to titrate the three standard solutions.

B_{std}= standard blank.

V_{sam}= sample volume in the flask (see section "Determination of Sample Volume").

V_r= volume of reagents used to fix the sample.

Tabla 5.1.
Table 5.1. Density of distilled water at a temperature t °C.

T (°C)	D (g cm^{-3})
20	0.99823
22	0.99777
24	0.99730
26	0.99679
28	0.99623
30	0.99567

Method 6
DETERMINATION OF pH

Yrene M. Astor

Introduction

This procedure describes the method to determine pH of seawater on the total hydrogen ion concentration (pH_T) pH scale, using spectrophotometric measurements developed by Clayton and Byrne (1993). The method is described in Dickson *et al*. (2007). The total hydrogen ion concentration is expressed in moles per kilogram of seawater. Spectrophotometric pH measurements are simple, fast and precise (Clayton and Byrne, 1993). The precision of the method is of ± 0.001 units of pH. The high levels of hydrogen sulfide in anoxic waters shorten the life of the electrodes used by other potentiometric methods; therefore, it is better to use a spectrophotometric method in this type of waters.

Principle of Analysis

The pH of a sample is determined by a spectrophotometric method adding a sulfonephthalein indicator, called *m*-cresol purple. The method is based on measuring the absorbance spectrum of the indicator, which has a p*K* value around the pH of seawater and the acid (HI^-) and basic (I^{2-}) forms of the indicator. The uncertainty level is equivalent to an imprecision in the total hydrogen ion concentration of ± 0.1% where

$$pH_T = -\log [H^+]_T \quad (1)$$

$$[H^+]_T \cong [H^+]+[HSO_4^-] \text{ (Byrne } et\ al.\text{, 1999)} \quad (2)$$

When spectrophotometric means are used in the analysis, the pH in the sample is determined from the following formula:

$$pH_T = pK_2 + \log_{10}\left(\frac{[I^{2-}]}{[HI^-]}\right) \quad (3)$$

where

pK_2 = dissociation constant for species HI^- (expressed on the scale of the total hydrogen ion concentration in mol kg-solution).

The ratio $[I^{2-}]$ / $[HI^-]$ is calculated by measuring the absorbance of both species at two wavelengths (434 and 578 nm). For each corresponding wavelength, the maximum absorbance of the basic and acid form of the m-cresol purple indicator is used to calculate the absorbance ratio ($R = A_{578}/A_{434}$) which describes the extension of the protonation of the indicator.

$$\frac{[I^{2-}]}{[HI^-]} = \frac{R_{(25)} - 0.00691}{2.222 - (R_{(25)} * 0.1331)} \quad (4)$$

where

$R_{(25)}$ = absorbance ratio (A_{578}/A_{434}) at 25 °C.

A_λ = corrected absorbances measured at wavelengths corresponding to the maximum absorbances of the basic (I^{2-}) and acid (HI^-), form, respectively.

Analytical Warnings

1. Temperature has influence on the spectrophotometric measurements of pH since it affects the formation constant of HI^- and molar absorptivities of the *m*-cresol purple indicator; therefore, perform the analysis at a temperature of 25 ± 0.1 °C.
2. The presence of air bubbles during sampling can alter the sample. Take the same precautions as with sampling dissolved oxygen (Method 5).
3. Yao *et al*. (2007) demonstrated that *m*-cresol purple has impurities that contribute to apparent deviations of the pH of up to 0.01 units of pH. Therefore, the analyst must maintain a record of the source and lot number of the indicator in order to be able to carry out corrections to the data once indicators with a degree of greater purification can be obtained and better characterizations can be performed.

Materials

1. Silicone Tygon® tubing for sampling from Niskin bottle, with a diameter less than 4 mm. This kind of tubing is used when samples for total and dissolved organic carbon are taken after pH. Soak the hose in clean seawater for 24 hours before the cruise to decrease the number of bubbles that adhere to the hose during sampling.
2. Optical glass cells, 10-cm light path, with Teflon® caps. Be sure to have enough cells for each sample and each replicate.
3. Gilmont micrometer syringe with an accuracy of 0.5%, with a thin Teflon® tube attached to the tip of the dispenser (Figure 6.1).

Equipment

1. Spectrophotometer with a bandwidth of 380-850 nm for spectral measurements.
2. Thermostatic sample compartment to accommodate cells of 10-cm where temperature can be regulated within ± 0.1 °C.
3. Water bath with thermocirculator with temperature control of ± 0.05 °C that regulates the temperature of the compartment as well as of the entire system.

Reagents

1. ***Solution of m-cresol purple (~ 2 mmol dm^{-3})***. Weigh 0.0809 g of *m*-cresol purple, sodium salt ($C_{21}H_{17}NaO_5S$, molecular weight: 404.41 g mol^{-1}) in 100 mL of distilled water. Adjust the pH of the solution to be in the range of 7.9 ± 0.1 pH units before each cruise.

Sampling

1. Draw the samples after collecting H_2S and dissolved oxygen. Adapt tubing to the tip of the Niskin bottle to capture the sample while avoiding air bubbles during sampling and thus diminishing this interference. Draw the sample directly into the cell.
2. Connect the tubing to the bottom inlet of the cell while keeping the cell in a vertical position, and allow water to flow in smoothly without bubbles. Flush three times with the sample while pressing the tubing with the fingers to eliminate air bubbles.
3. Place the cell horizontally and allow the water to overflow at least two volumes of the cell. This can be estimated by counting while cell fills.
4. While the flow continues, carefully place the Teflon® cap in one of the openings of the cell without trapping air bubbles inside. Remove the hose and place the second Teflon® cap.

5. Store the cell in the dark at room temperature while waiting analysis. Analyze as quickly as possible (less than 30 min).

Sample Analysis

Turn on the equipment 15 minutes before beginning the analysis so it stabilizes.

1. Place each cell containing the sample in a Ziploc® bag and put inside a thermostatic bath to 25 °C for 15 minutes.
2. Clean and dry the exterior of the cell and place it in the thermostatic compartment at 25 °C.
3. Calibrate the spectrophotometer with the sample and add 20 µL of *m*-cresol purple indicator to the cell with a microsyringe. Replace the cap trying to avoid trapping air bubbles and mix the sample with the indicator.
4. Measure the absorbances at three wavelengths: 730 nm corresponding to a non-absorbing wavelength of m-cresol purple, and 578 and 434 nm, which correspond to the maximum absorbance of the base (I^{2-}) and the acid (HI^{-}) of the indicator respectively.

Calculation and Expression of Results

The pH of seawater is obtained through the following formula:

$$pH_T = pK_2 + \log_{10}\left[\frac{R_{(25)} - 0.00691}{(2.222 - (R_{(25)} * 0.1331))}\right] \qquad (5)$$

where

pK_2= dissociation constant for HI^- species (expressed on the total hydrogen ion concentration pH scale in mol kg-soln).

$R_{(25)}$= absorbance ratio ($R = A_{578}/A_{434}$) measured at 25 °C.

Dissociation constant K_2 for *m*-cresol purple depends on salinity and temperature and it is obtained from the formula in Clayton and Byrne (1993):

$$pK_2 = \frac{1245.69}{T} + 3.8275 + F + (0.00211*(35 - S)) \qquad (6)$$

where

T = water bath temperature, in degrees Kelvin.
S = sample salinity.
F= correction according to DelValls and Dickson (1998): 0.0047.

The correction factor arises because addition of the indicator alters the pH. Therefore, it is necessary to calculate a correction factor to obtain better results (see Dickson *et al.*, 2007).

The absorbance ratio $R_{(25)}$ is obtained from the following formula:

$$R_{(25)} = \frac{(A_{578I} - A_{730I})}{(A_{434I} - A_{730I})} \qquad (7)$$

where

$A_{\lambda I}$ = absorbances of seawater with *m*-cresol purple indicator at wavelength 434, 578 and 730 nm.

Method 7
DETERMINATION OF TOTAL ALKALINITY

Yrene M. Astor

Introduction

This procedure describes the method for the determination of total alkalinity in seawater using spectrophotometric measurements, according to Breland and Byrne (1993) and Yao and Byrne (1998), modified according to suggestions of Dr. Wensheng Yao. The results are expressed in µmol kg^{-1} of seawater. The precision of the method is ± 2 µmol kg^{-1} and it is suitable for oceanic levels of total alkalinity between 2000-2500 µmol kg^{-1}.

Principle of Analysis

A known volume of seawater is acidified with a solution of 0.1N HCl to a pH between 3.8 y 4.2. The content of CO_2 is purged from the acidified sample using nitrogen gas. The excess of acid is quantified by means of spectrophotometric measurements using a sulfonephthalein indicator, bromocresol green. The absorption maxima for the species I^{2-} (616 nm) and HI^{-} (444 nm) are the ones used in the spectrophotometric measurements of alkalinity.

Analytical Warnings

1. Temperature influences alkalinity spectrophotometric measurements since it affects the formation constant of HI^{-} and the molar absortivities of the sulfonephthalein indicator; therefore, the analysis must be performed to a constant temperature of 25 ± 0.1 °C.

Materials

1. Cells of 10-cm light optical glass path with Teflon® caps.
2. Syringe, micrometer with an accuracy of 0.5%, calibrated to 2 mL, with thin Teflon® tubing adhered at the tip to dispense the indicator. (Figure 6.1).
3. Amber borosilicate bottles, 250 mL, previously washed with detergent and deionized water.
4. Plastic syringe, capacity 5 mL, with a thin Teflon® tubing adhered to the tip.
5. Tygon® tubing for sampling from the Niskin bottle, with a diameter no greater than 4 mm. It is necessary to immerse the tubing in filtered seawater for 24 hours before the cruise to minimize the quantity of bubbles that stick to the hose during sampling.

Equipment

1. Double beam spectrophotometer for spectral measurements with a bandwidth of 380–850 nm.
2. Analytical balance, with accuracy of 0.001 g.
3. Thermostatic compartment to accommodate cells of 10-cm where the temperature could be regulated inside to a range no greater than ± 0.1 °C.
4. Water bath with termocirculator with temperature control of ± 0.05 °C that regulates the temperature of the compartment as well as of the whole system.
5. Magnetic agitators.

Reagents

1. ***Saturated mercury chloride***.
2. ***Bromocresol green indicator (~ 2 x 10^{-3} M).*** Weigh 0.216 g of bromocresol green, sodium salt ($C_{21}H_{13}Br_4NaO_5S$, molecular weight: 720.06 g mol^{-1}) and dilute to 100 mL with distilled water. Adjust the pH of the solution to 4.5.
3. ***Hydrochloric acid, (0.1 N)***, Dilute 8.3 mL of hydrochloric acid (38% HCl) in distilled water. Complete to one liter.
4. ***High purity Nitrogen gas***.
5. ***Reference material for measurements of oceanic CO_2.*** Supplied by Dr. Andrew G. Dickson of Marine Physical Laboratory (co2crms@ucsd.edu)

Sampling

1. Connect the Tygon® tubing to the mouthpiece of the Niskin bottle, put the tubing in the bottom of the amber bottle, wash with the sample three times and fill allowing the water overflows the bottle. Calculate the volume of water that should overflow by estimating the time the bottle takes to be filled in and then allow water to continue overflowing for a period equivalent to twice that time.
2. Raise the tubing near the mouth of the bottle, close the spigot and withdraw the tubing. This technique leaves an air space in the bottle of approximately 2.5 mL (1% of the total volume of the bottle) which allows the expansion of the water for changes in temperature.
3. Add with an automatic pipette 50 µL (0.02% of the total volume of sample) of a solution of saturated mercury chloride in order to prevent biological activity. Wear gloves while adding the reagent.
4. Close the bottle and mix the solution.
5. Keep refrigerated, not frozen. Replicates of three different depths are taken from each station. Samples can be refrigerated for no more than three months.

Sample Analysis

Turn on all the equipments 15 minutes before beginning the analysis to stabilize. Check the pH of the indicator (< 4.5) and calibrate the analytical balance. Allow the samples, standard solution and reagents reach room temperature before the analysis. During the manipulation of the flasks and the syringes, hands must remain clean and dry. The following procedure is performed to the samples and to the standard solutions:

Measurement of absorbances before titration

1. Place the cell with the sample (or standard solution) in a Ziploc® bag and put inside a thermostatic bath at 25 °C for 15 minutes.
2. Dry the exterior of the cell and place it in the thermostatic compartment at 25 °C.
3. Measure the absorbances at the following wavelengths: 444, 616 and 750 nm.

   NOTE. Use the same cell to measure the absorbances before and after the titration. Replicates should be measured in the same cell.

Titration

1. Weigh with a precision of 0.01 g enough empty flasks of 125 mL according to the number of samples and standard solutions to be measured.
2. Add 100 mL of sample (or standard solution) to a flask with a volumetric pipette placing the tip of the pipette at the bottom of the flask.

3. Weigh the flask with the sample (or standard solution). The difference in weight between the flask with sample and the empty flask represents the sample volume (V_s).
4. Add with a micrometer syringe 80 µL of bromocresol green indicator while mixing.
5. Weigh with a precision of 0.001 g a plastic syringe with 0.1N HCl, and titrate.
6. Add acid to the sample slowly while mixing until a green yellowish color (pH between 3.8 and 4.2) appears. Place the tip of the dispensing tubing close to the surface of the liquid trying that the acid falls without splashes assuring the total mixture of the acid with the sample.
7. Weigh again the syringe. The difference of weight before and after the titrations represents the volume of the acid added (V_a).
8. Continue mixing while supplying nitrogen gas for 5 minutes.

Measurement of absorbances after titration

1. Fill a 10-cm cell with the sample and place inside a Ziploc® bag and place it in a thermostatic bath to 25° C for 15 min.
2. Dry the exterior of the cell and place it in the thermostatic compartment at 25 °C.
3. Measure the absorbances to the following wavelengths 444, 616 and 750 nm while the temperature is kept to 25 °C.

Calculation and Expression of Results

The absorbance measured to 750 nm is used to control and correct any diversion of the base line. The way the cell is placed in the thermostatic compartment as well as the drift of the equipment can make changes in the base line; therefore, a measurement is made at a wavelength where no absorption occurs (750 nm for bromocresol green). This absorbance is subtracted from the absorbance measured for the samples (or standard solutions). The difference between the absorbance before and after the titration at 750 nm should not be greater than ± 0.001.

The total alkalinity is calculated using the following equation:

$$(AT)_s = \frac{\{(M_a * V_a) - [(H^+)_T * V_{sa}]\} * 1000}{V_s} \quad (1)$$

where

$(AT)_s$ = total alkalinity of the water sample in µmol kg^{-1}.

M_a = concentration of HCl added during titration.

$$M_a = \left(10^{-pHsws} * \frac{(V_s + V_a)}{V_s}\right) + AT_{std} + \frac{V_s}{V_a} \quad (2)$$

V_s = volume (or mass) of the seawater sample before titration.

V_a = volume (or mass) of the added acid.

V_{sa} = volume (or mass) of the seawater after titration.

AT_{std} = total alkalinity of the standard solution (Marine Physical Laboratory) according to standard certificate.

$(H^+)_T$ = total concentration of the excess of ions of hydrogen in the seawater sample after removing completely the CO_2.

$$(H^+)_T = [(10^{-pHsws} + [HI^-])] \quad (3)$$

where

$$(H^+)_T = [H^+]_T + [HI^-] \quad (4)$$

$$[H^+]_T = [H^+] + [HSO_4^-] + [HF] = 10^{-pHsws} \quad (5)$$

$$pH_T = 4.2699 + 0.002578*(35-S_a) + \log \left[\frac{(R - 0.00131)}{(2.3148 - (0.1299)*R)} \right] \quad (6)$$

$$pH_{sws} = pH_T - \log (1 - (0.001005 * S_a)) \quad (7)$$

where

pH_T = pH in the pH total scale.
pH_{sws} = pH in the seawater pH scale.
S_a = salinity of the solution after titration:

$$S_a = \frac{(S_s * V_s)}{(V_a + V_s)} \quad (8)$$

where

S_s= salinity of the sample before adding the acid.

$$R_{(25)} = \left[\frac{(A_{616I} - A_{750I}) - (A_{616wi} - A_{750WI})}{(A_{444I} - A_{750I}) - (A_{444wi} - A_{750WI})} \right] \quad (9)$$

where

$R_{(25)}$ = quotient of absorbances (R= 616A / 444A). $R = A_{616} / A_{444}$.

$A_{\lambda I}$ = absorbances at λ = 444, 616 and 750 nm with indicator.

$A_{\lambda WI}$ = absorbances at λ = 444, 616 and 750 nm without indicator.

Method 8

DETERMINATION OF SPECTRAL ABSORPTION COEFFICIENTS OF THE PARTICLES AND PHYTOPLANKTON

Laurencia Guzmán and Ramón Varela

Introduction

The size, nature and abundance of suspended particles in seawater has influence on the light climate in the water, which has a direct effect on the phytoplankton activity and distribution, and indirectly on the concentration of some chemical elements found in the photic layer. On the other hand, the inherent optical properties can reveal the characteristics and amount of particles in seawater. The following procedure describes the method to determine the spectral absorption coefficient for particles as well as for only phytoplankton. The technique measure the light absorption that goes through a wet filter that contains the particles (Kishino *et al.,* 1985).

Principles of Analysis

The method is based on the change observed in the spectrum of light absorption of a wet filter that retains the suspended particles of a seawater sample with respect to a reference filter (blank). The absorption spectrum is measured for the filter with the sample before and after the extraction of the pigments with hot methanol (60 ºC). This technique determines the total absorption particle coefficient, $a_p(\lambda)$, and the detritus absorption coefficient (particles without their pigments), $a_d(\lambda)$. The difference in the optical transmission between these two parameters is related to the absorption coefficient of phytoplankton, $a_{ph}(\lambda)$. The results are expressed in m^{-1}.

Analytical Warnings

1. In the measurements, variations occur due to differences in the characteristics of particles in the water, the kind of equipment used (spectrophotometer or espectroradiometer) and the type of filter. This interference is corrected in particular for each case applying a pathlength amplification factor, ß (Kishino *et al*., 1985; Mitchell and Kiefer, 1988; Bricaud and Stramski, 1990; Mitchell *et al.,* 2002).

2. If the extraction of pigments with the solvent is incomplete, the coefficient of the detritus material can be overestimated and this will affect the absorption coefficient of the phytoplankton. It is important to observe that there is no phytoplankton absorption at longer wavelengths (red).

3. The amount of material retained by the filter should not generate an optical density (OD) greater than 0.25 and lower than 0.05 when measured at 675 nm, or lower than 0.4 at 440 nm (Mitchell et al 2002). On the other hand, when the amount of material retained by the filter is too low, it is difficult to observe a change of color in the surface of the filter. To control the intensity of the color in the filter, the volume of water filtered must vary according to the amount of particles present in the sample.

Materials

1. Filters Whatman, GF/F, nominal 0.7 µm, 25 mm diameter.
2. Small Petri dishes (plastic).
3. Plastic syringe, 60 mL.
4. Tubing of 10 cm in length that is connected to the end of the syringe.
5. Drop Pasteur pipette with rubber bulb, 5 mL.
6. Funnel for filters of 25 mm diameter and with a capacity of 200 mL.
7. Graduated Cylinder, 50 mL.

Equipment

1. Filtration unit and vacuum pump for sampling as well as for the laboratory.
2. Measuring equipment
   a. Spectrophotometer with a bandwidth of 380-800 nm for spectral measurements, and an optical resolution of the instrument lower than 4 nm. In order to be able to measure the OD of the filters in a spectrophotometer, a special accessory is required (but not available from all manufacturers) that illuminates the totality of the area of the filter for light transmission and that measures the light that goes through it.
   b. Another option is to use a spectroradiometer. This type of instrument measures the radiometric amounts in intervals of narrow wavelengths for given spectral regions. The equipment uses an optical fiber with a narrow angle of view that focuses on the total area of the filter. The filter is illuminated totally and uniformly with a halogen incandescent lamp. In the analyses of the CARIACO project, a Spectrascan PR-655 from Photo Research is used. Also, a dark box is used with a light source that holds and illuminates the filter by transmission, and serves as a fixed support to focus the optical fiber (Figure 8.1). This kind of equipment receives the incident light beam and spreads different wavelengths in different angular directions after the light goes through the sample. The sensible element is a series of photodiodes that measure the different wavelengths. The useful range of these measurements is between 420 and 750 nm, since the shorter wavelengths are detected at very low intensities and the optical fiber that is used can absorb them strongly.
3. Water bath up to 60 °C with a stability of ± 0.5 °C.
4. Measure the amount of chlorophyll present in the methanol extract that has washed the filter, using a spectrophotometer with 10-cm cells, following the procedure for spectrophotometric pigment analysis (Strickland and Parsons, 1972), or use a fluorometric technique to measure chlorophyll following **Method 13** of this manual.

Reagents

1. **Methanol** (CH_3OH).

Sampling

1. Draw water samples from the rosette into dark plastic 2 L bottles.
2. Filter the water through Whatman GF/F filters using a vacuum pump. Record the filtered volume. The volume to filter depends on the amount of particles in the water, which is indicated by the degree of saturation of the filter. In waters with moderate biomass (0.5 to 1 mg m^{-3} of chlorophyll), which is typical of waters near the coasts, an average volume

of 2 L is enough. If water has more particles, reduce the volume to as little as 0.25 L. In oceanic waters, increase the volume until an appreciable coloration is produced on the filter.

3. Store each filter without folding in a Petri dish properly identified, with the surface that retains particles upwards.
4. Cover the dish with aluminum foil to avoid exposure to light.
5. Freeze the filters placing the dish horizontal until just before the analysis.

Sample Analysis

Turn on the lamp 15 minutes before beginning the analysis so it stabilizes.

1. ***Sample preparation***

   a. Let the filters with the samples reach room temperature.

   b. Place absorbent paper, dampened with distilled water, below the filters inside the Petri dish to keep them moist. Use a spatula to raise the filter without touching its upper face, and keep the dish covered with aluminum foil all the time.

   c. Prepare a reference filter (blank) in the same way as in step b.

2. ***Analysis (using a spectroradiometer)***

   a. ***Absorption of light by all particles (a_p):***

      i. Measure the radiance of the blank filter (Lb).
      ii. Measure the radiance of the filter with the sample (Ls).
      iii. Optical density is obtained with the formula:

$$OD_s(\lambda) = \log_{10}\left(\frac{Lb\ (\lambda)}{Ls\ (\lambda)}\right) \qquad (1)$$

NOTE. When using a spectrophotometer, the optical density (OD) is obtained directly.

   b. ***Absorption due to detritus material a_d, (extraction with hot methanol).***

      i. Place methanol in a bottle with the cover slightly open.
      ii. Warm up methanol in a water bath to a temperature of 60ºC. **Wear gloves and safety glasses and work in a chemical fume hood during this part of the analysis.**
      iii. Place the filter with sample in a filtration funnel (Figure 8.2).
      iv. Add hot methanol to the filter in aliquots of 5 mL using a Pasteur pipette with a rubber bulb.
      v. Filter slowly while adding the methanol until 50 mL of methanol are used. Recover the filtered solution in a graduated cylinder under the filter holder (Figure 8.2).
      vi. Record the volume filtered into the cylinder. This volume can be used to determine chlorophyll concentration.
      vii. Measure the radiance of the blank filter (Lb).
      viii. Measure the radiance of the filter with the sample after being washed with hot methanol (Lws).
      ix. Calculate the new optical density $OD_{ws}(\lambda)$ with formula (1).

To determine the amount of chlorophyll *a* or calculate the pigment indexes by spectrophotometry and/or fluorometry (Strickland and Parsons, 1972), begin by mixing the extract of pigments in methanol by pumping with a syringe to which a small hose is attached at one

end. This syringe is used to decant the extract to any recipient adequate for the measurements.

Calculation and Expression of the Results

The absorption coefficient for the visible band from 380 to 750 nm, with a resolution less than 4 nm, can be calculated by the following expressions:

1. The total particle absorption is calculated with the following equation:

$$a_p(\lambda) = A^* \left[\frac{(2.3 * OD_p(\lambda))}{(ß * V)} \right] \quad (2)$$

where

$a_p(\lambda)$ = total absorption particle coefficient (m^{-1}).

A = filter used area = 3.63 x 10^{-4} m^2 for 25 mm filters.

$OD_p(\lambda)$ = corrected optical density = $OD_S(\lambda)$-OD(750).

OD(750)= Adjustment = average OD between 752 nm and 780 nm, where phytoplankton absorption is assumed to be zero.

ß = 1.63 $[(OD_p)^{-0.22}]$ (filter amplification factor; Mitchell and Kiefer, 1988, Bricaud and Stramski, 1990).

V = volume of filtered water in m^3.

2. The absorption coefficient of detritus material is calculated with the following equation:

$$a_d(\lambda) = A^* \left[\frac{(2.3 * OD_d(\lambda))}{(ß * V)} \right] \quad (3)$$

where

$a_d(\lambda)$ = detritus absorption coefficient due to vegetal remains and other particles without chlorophyll (m^{-1}).

$OD_d(\lambda)$ = corrected optical density = $DO_{ws}(\lambda)$ – OD(750)·

ß = 1.63 $[(OD_d)^{-0.22}]$

The same calculations are used as for $a_p(\lambda)$, but using the absorbances of the filter after the extraction with hot methanol

3. The phytoplankton absorption coefficient is calculated with the following equation:

$$a_{ph}(\lambda) = a_p(\lambda) - a_d(\lambda) \quad (4)$$

where

$a_{ph}(\lambda)$ = light absorption coefficient due to phytoplankton pigments (m^{-1}).

Method 9

DETERMINATION OF COLORED DISSOLVED ORGANIC MATTER

Laurencia Guzmán and Ramón Varela

Introduction

Colored dissolved organic matter (CDOM) is the result of the decomposition of organic compounds, mainly of plant origin, contributed by the rivers and the degradation of phytoplankton in the ocean. CDOM is defined as the material that goes through a filter of 0.2 µm, and it is quantified in units of absorbance (m^{-1}) for specific wavelengths. The absorption spectrum of CDOM, with an absorption maximum in the ultraviolet, exponentially decreases towards longer wavelengths. This procedure describes the method to determine CDOM using a spectrophotometric method in which the absorption coefficient of the dissolved material (a_g) is calculated based on Bricaud *et al.* (1981), Nelson and Siegel (2002) and Nelson *et al.* (2007).

Principle of Analysis

The absorbance of CDOM is measured in the ultraviolet and blue regions using a spectrophotometer. The absorption coefficient of the dissolved material is calculated from these values for desired wavelengths.

Analytical Warnings

1. Use recently distilled water for the blank and store in a refrigeratator in the dark to prevent any alteration. A contaminated blank can affect the absorption coefficient of CDOM.
2. A fluctuating baseline can affect the absorption coefficient of CDOM; therefore, the baseline on the spectrophotometric measurements must be constant.
3. Filter the sample slowly to avoid breaking the filter (which allows particles to go through) or cell rupture on the filter (releasing intracellular components).
4. The exposure of the samples to light over long periods can result in a lost of color which affects the absorption coefficient of CDOM.

Materials

Wear polyethylene gloves all the time while handling samples and materials.

1. Nylon syringe filters, 25 mm in diameter and pore size 0.2 µm.
2. Plastic syringes without black rubber plungers (Figure 9.1).
3. 60 mL glass syringe with stainless steel laboratory pipetting needle (10.2 cm length, Figure 9.2).
4. Quartz cells, 10-cm pathlength, with Teflon® caps.
5. 60 mL amber glass bottles with Teflon® caps.

Reagents

1. ***Hydrochloric Acid (HCl),* 10%.**
2. ***Ethanol (CH_3OH), high reagent grade or better***.

Equipment

1. Spectrophotometer with a bandwidth of 200-850 nm for spectral measurements. It is conveniente to perform a run with a 2 nm resolution.

Sampling

1. ***Pre-treatment of sampling material***
   a. Soak the amber bottles, their caps and the syringes in a solution of 10% HCl overnight at room temperature, then rinse with distilled water.
2. ***Sampling***
   a. Before sampling, remove the plunger from the plastic syringe and wash the syringe three times with the sample. Use the syringe to capture the sample while wearing polyethylene gloves.
   b. Connect the nylon filter to the tip of the syringe and fill it up. Filter the sample directly into the amber bottle. Rinse the amber bottle three times with the filtered sample and fill. Allow an air space in the bottle of approximately 1 mL (10% of the total volume of the bottle) for the expansion of the water when it freezes.
   c. Store the sample at -20 °C.

Sample analysis

1. Suitable preservation of the sample allows storage without alteration for several months, as long as it remains frozen and protected from light.
2. Defrost the samples and allow them to reach room temperature.
3. Rinse the syringe, the pipetting needle and the cells with recently distilled water and ethanol.
4. Extract a sample from the bottle using a glass syringe with a pipeting needle.
5. Remove the needle and place a syringe filter of 0.2 µm on the syringe. Filter directly into the quartz cell rinsing three times with the sample. Fill the cell completely without leaving any bubbles. Clean and dry the exterior of the cell perfectly.
6. Prepare a cell with recently distilled water (blank) at room temperature.
7. Measure the absorption spectra of the sample (absorbance, A(λ)) between 230 and 700 nm, zeroing the spectrophotometer with the blank. After finishing the measurement, samples should not be refrozen, but they can be refrigerated in their amber bottles for up to 3 to 4 days.
8. If it is necessary, correct fluctuations of the baseline by subtracting the absorbance value at the wavelength of 700 nm.

Calculation and Expression of Results

The calculation of the absorption coefficient of dissolved matter (a_g) is made using the following expression (Bricaud *et al*., 1981)):

$$a_g(\lambda) = 2.303\, A(\lambda)/L \qquad (1)$$

where:

$A(\lambda)$ = absorbance at a determined wavelength (m^{-1}).
L = cuvette cell length (0.1 m).

Method 10

DETERMINATION OF TOTAL AND DISSOLVED NITROGEN AND ORGANIC CARBON

Laura Lorenzoni

Introduction

This procedure describes the method to determine total organic carbon (TOC), dissolved organic carbon (DOC), and total and dissolved nitrogen (TN and TDN) in seawater. TOC is the total content of carbon in seawater found in nonvolatile organic compounds. DOC is the fraction of the TOC that goes through a filter with pore diameter of 0.7 µm where the inorganic carbon is eliminated by acidification and aeration. TN is the sum of all nitrogen forms (inorganic and organic), and TDN is the dissolved fraction of total nitrogen in seawater, defined in the same way as the DOC. This determination uses a catalytic combustion method. The results are expressed in µmol L^{-1} of carbon (nitrogen). The range of concentrations detected by this method is between 0 and 400 µmol L^{-1} of dissolved organic carbon and between 0-50 µmol L^{-1} of total dissolved nitrogen. The method described is taken from Dickson *et al.* (2007).

Principle of Analysis

The method for TOC (DOC) is based on the total oxidation of nonvolatile organic compounds to CO_2 through catalytic combustion to 680 °C. The seawater sample, filtered for DOC (and unfiltered for TOC), is acidified and sparged with oxygen before combustion. The sample is injected onto a combustion column packed with platinum-coated alumina beads held at 680 °C. The amount of CO_2 produced during this process is measured with a Nondispersive Infrared Detector (NDID). For the analysis of TN (TDN), the sample is calcinated to convert the nonvolatile nitrogen compounds into NO forming chemiluminescence when mixed with ozone, which is measured with a photomultiplier.

Analytical Warnings

1. The inorganic carbon present in the sample produces a positive interference, which is eliminated when the sample is acidified and sparged with oxygen before the combustion.
2. Contamination of the sample can occur easily through air, contact with fingers or by touching the spout of the Niskin bottle without wearing gloves. It is necessary to be careful during sampling, handling, filtration and storage of the sample to prevent contamination. Some precautions are:
   a. Wear polyethylene gloves all the time when collecting TOC and DOC samples as well as with those samples taken before TOC or DOC (like gases).
   b. During sampling, only silicone hoses must make contact with the spout of the Niskin bottle, since any other type as Tygon® represents a source of contamination. Use silicone hoses with previous samples as well.
   c. Avoid contact of the filter holder or the spout of the Niskin bottle with the polyethylene bottle used to capture the sample.

d. Avoid contact with grease.
e. Store the sample in a freezer that does not contain, at present or previously, organic material or solvents (such as food, biological samples, etc.).

3. Manipulation may be a source of contamination during the filtration process. It is better not to filter the samples with a low content of particulate organic carbon (< 1 µmol L^{-1}); however, filtration is necessary in samples with a high particle content (like in highly productive waters). In the Cariaco Basin, samples from depths above 250 m are filtered (see Table 1.3, **Method 1** for sampling depths) due to the high content of particles that are found in superficial waters, as well as near the interface where bacteria accumulate.

Materials

1. Filters Whatman GF/F, 47 mm diameter, calcinated during four hours at 450 °C and stored in closed glass petri dishes (Figure 10.1).
2. Silicone hoses and polycarbonate inline 47 mm filter holders, washed in 10% HCl solution and rinsed three times with distilled water (Figure 10.2).
3. High Density Polyethylene (HDPE) bottles, capacity 60 mL, soaked in a 10% HCl solution for four hours, and rinsed three times with distilled water. Dry upside down on a clean surface.

Reagents

1. ***High purity (99.995%) oxygen gas***.
2. ***Combustion column catalyst.*** Column packed with 2 mm platinum-coated alumina beads (Shimadzu P/N 017-42801-01) held at 680 °C.
3. ***Platinum gauze.*** Pure platinum wire gauze (52 mesh, woven from 0.1 mm diameter wire), roughly formed into cubes (~ 0.5 cm to a side). Three to five cubes are placed at the top of the combustion column bed.
4. ***Consensus Reference Material (CRM).*** Deep Sargasso Sea/Florida Straight water (44-46 µM DOC), available in 10 mL ampoules from the Department of Marine and Atmospheric Chemistry, University of Miami, RSMAS/MAC (http://www.rsmas.miami.edu/groups/biogeochem/CRM).
5. ***Low Carbon Water (LCW).*** Water with a carbon concentration between 1-2 µmol C L^{-1}, which is also obtained from RSMAS/MAC.
6. ***Hydrochloric acid (10% HCl).***
7. ***Hydrochloric acid (HCl).*** Trace metal analysis grade.
8. ***Potassium hydrogen phthalate*** **($C_8H_5O_4K$).** Prepared according to the carbon concentration in the sample.
9. ***Potassium nitrate*** **(KNO_3).**
10. ***Agua Milli-Q® water.***

Equipment

1. Organic carbon analyzer Shimadzu TOC-V_{CSH} with autosampler ASI-V and total nitrogen detector TNM-1 (or equivalent). The carbon and nitrogen detectors function independently. If another type of instrument is used, perform an evaluation of its application in seawater.

Sampling

1. Samples are taken after sampling gases and pH. First, the sample for TOC (TN) is collected, followed by the DOC (TDN) sample.
2. TOC sample is collected directly from the spout of the Niskin bottle. Rinse the HDPE bottle three times with the sample and fill the bottle ¾ full. Freeze in a vertical position (Figure 10.3).

3. The DOC sample is collected by connecting an inline filter holder to the spout of the Niskin bottle using a flexible silicone hose. The sample is filtered through a calcinated fiber glass filter placed within the holder. Allow the water to flow about two minutes through the inline filter holder and the filter. Rinse the HDPE bottle three times with the sample and then fill the bottle ¾ full. Freeze in a vertical position (Figure 10.4).
4. Between casts, disassemble the filter holders and submerge them in 10% HCl. Rinse several times with distilled water before assembling the holders for the following filtration. If enoug filtration units are prepared for all the samples taken in a cruise, this step is not necessary.

Sample Analysis

1. ***Sample pretreatment***

   a. Allow the samples reach room temperature.

   b. Reduce the pH to < 2 by acidifying the sample with concentrated HCl (trace metal analysis grade) in a volume estimated as 0.1% of the sample total volume.

2. *Analysis*

   a. The conditions for the organic carbon analyzer Shimadzu TOC-V_{CSH} are as follow:

Combustion temperature	680 °C
Carrier gas	High purity oxygen
Carrier flow rate	150 mL min^{-1}
Ozone generation, gas zero	Air from Whatman Gas Generator
Ozone flow rate	500 mL min^{-1}
Sample sparge time	2 min
Minimum number of injections	3
Maximum number of injections	5
Number of washes	2
Standard deviation maximum	0.1
CV maximum	2%
Injection volume	100 µL

   b. For DOC and TOC, the system is calibrated using a solution of potassium hydrogen phthalate, while for TN and TDN, the system uses potassium nitrate. Both solutions are prepared in Milli-Q® water.

   c. The performance of the equipment is verified before beginning the analysis, by using reference material (CRM). Low carbon water (LCW) also is employed to verify the operation of the apparatus.

   d. Place the samples in an auto-sampler for analysis. The run begins with a blank of Milli-Q® water and a CRM, followed by six samples, another blank of Milli-Q® water and a CRM. The sequence is repeated until all samples have been analyzed. The analysis finishes with a blank of Milli-Q® water, a CRM and a blank of Milli-Q® water that has not been acidified. This last blank allows verification of whether the acid used during acidification shows any sign of contamination. The blanks of Milli-Q® water and the CRM are used to determine the performance of the instrument

during the analysis. If a problem is detected, analyze the samples again.

Calculation and Expression of Results

1. ***Calculation of TOC or DOC.*** Calibrate the equipment Shimadzu TOC-V_{CSH} for carbon using an analysis of 4 to 5 injections of a standard solution of potassium hydrogen phthalate prepared with Milli-Q® water. The instrument determines the concentration in parts per million (ppm). The following formula is used to calculate the concentration in µmol L^{-1} of carbon corrected with the blank of the instrument:

$$TOC\ (DOC) = [(Sample_{(ppm)} - LCW_{(ppm)}) * 83.33333] + LCW\ value\ (\mu M) \quad (1)$$

where

Sample = carbon concentration of the sample determined by the analyzer.

LCW = carbon concentration of the low carbon water determined by the analyzer.

83.33333 = conversion factor from ppm to µmol L^{-1}.

When $LCW_{(ppm)}$ is subtracted at the beginning of the equation, it eliminates the instrumental blank as well as the carbon content of the LCW; therefore, the carbon content of the LCW is added again (term at the end of the equation) to calculate the correct concentration of the sample.

2. ***Calculation of TN or TDN.*** The calibration of the instrument is similar to the one for carbon. The standard solution used is potassium nitrate prepared with Milli-Q® water. The instrument is calibrated in ppm and the conversion to µmol L^{-1} is as follow:

$$TN\ (TDN) = Sample_{(ppm)} * 71.43 \quad (2)$$

where

Sample = nitrogen concentration of the sample determined by the analyzer.

71.43 = conversion factor from ppm to µmol L^{-1}.

There is no blank for the nitrogen system. Dissolved organic nitrogen (DON) can be calculated subtracting the inorganic nitrogen (NO_2^-, NO_3^-, NH_4^+) from the TDN determined by the analyzer.

Method 11

DETERMINATION OF PARTICULATE CARBON AND NITROGEN

Eric Tappa

Introduction

This procedure describes the method for determining particulate carbon and nitrogen (PC and PN) in seawater. The PC (PN) is the total content of carbon (nitrogen) in seawater that does not pass through a filter of known porosity (0.7 µm) and therefore is retained in the filter. As the organic matter present in seawater can be found both dissolved as well as in particles, the particulate organic carbon (POC) and particulate organic nitrogen (PON) are defined as the organic fraction of the material retained by the filter. The procedure quantifies the amount of carbon (nitrogen) in the filter through an elemental analyzer, which uses a method of dry combustion in a helium/oxygen atmosphere in which the carbon (nitrogen) is reduced to CO_2 (N_2 gas). The results are expressed in µg L^{-1} of carbon (nitrogen). The concentration range of the method is between 5 and 500 µg C L^{-1} and 0.5-100 µg N L^{-1}. In the samples of the Cariaco Basin, the inorganic fraction is not significant; therefore, it is considered that the material retained by the filter is mainly of organic origin. The method described below is taken from Sharp (1974).

Principle of Analysis

The method for PC (PN) is based on the dry combustion (960 °C) of organic and inorganic substances present in the sample in an atmosphere of pure helium in which oxygen is added as a catalyst. The gases from the combustion are carried by UHP helium into a chromatographic column where they are separated and then quantified by a thermal conductivity detector.

Analytical Warnings

1. If the objective is to establish the concentration of particle organic carbon, the inorganic carbon fraction (carbonates and bicarbonates) in the sample must be eliminated. The removal process is done by exposing the filters that contain the sample to HCl fumes (Schubert and Nielsen, 2000) in a desiccator for 24 hours where an open container with acid is placed in the lower compartment of the desiccator. Later, the filters are dried at 65 °C for 24 hours, folded inside tin discs, and stored in a desiccator until analysis.
2. The presence of organic material or oil on the filter, disks and plates can also contaminate the sample. Filters must be pre-combusted, and tin disks and petri dishes should be washed with methanol before using them. Use polyethylene gloves at all times while manipulating the filters, disks and petri dish.

Materials

1. Fiberglass filters, Whatman GF/F, 25 mm in diameter. Pre-combust filters for four hours at 450 °C, then immerse in 10% HCl for 4 ½ hours, then submerge in distilled water for two hours. Place filters in an oven at 70 °C over night and store in petri dishes.

2. Small petri dishes. Immerse in methanol and place in a desiccator for an hour. Put a piece of weighting paper in each dish.
3. Filtration unit and vacuum pump for sampling.
4. Dark plastic bottles of 2 L.
5. Tweezers, combusted and washed with methanol.
6. Tin disks (for filters and standards) of 30 mm in diameter. Wash with methanol and place in an oven at 60 °C for one hour.
7. Desiccators.
8. Teflon® tubes.
9. Funnels for 25 mm filters and 200 mL of capacity.

Equipment

1. Elemental Analyzer CHN Perkin Elmer 2400, instrument precision for carbon of 0.2% and 0.1% for nitrogen.
2. Microbalance with a precision of 0.001 g.
3. Oven (103–105 °C).

Reagents

1. ***Helium gas*.** Ultra high purity.
2. ***Oxygen gas*.** Ultra high purity.
3. ***Cystine.*** Quality reagent (29.99% C and 11.66% N).

Sampling

1. Collect samples from the Niskin bottle in plastic dark bottles of 2 L, washing them three times with the sample before filling.
2. Filter through pre-combusted filters Whatman GF/F. Record the volume filtered. The volume filtered depends on the amount of particles in the water, which is indicated by the degree of filter saturation. In coastal waters, an average volume of 2 L is enough. If the water have a high concentration of particles, reduce the volume up to 0.25 L. Ocean waters requires a larger volume (~ 4 L).
3. Store the filters in a pre-washed petri dish, label and freeze. Store three blank filters for each cast.

Sample Analysis

1. ***Sample pretreatment***

   a. Place the petri dish and its filter, uncovered, in an oven at 60 °C over night to thaw and dry.
   b. Place the filter with the sample side down on a tin disk, roll and fold the ends using two precombusted tweezers.

2. ***Analysis***

   a. Place the filter and the tin disk in a Teflon® tube and compress with a Teflon® rod.
   b. The samples are then introduced via an autocarousel into an Elemental Analyzer PE2400 according to the manufacturer's instructions.

Analysis of Standard Solutions and Blanks

1. Blanks are analyzed to determine baseline levels of C and N. Place the blanks in tin disks, roll and fold the ends like the samples. Perform the analysis according to the procedure specified in section **Sample Analysis**.
2. Calibrate the PE 2400 with Cystine crystals as standard. Weigh the cystine (between 0.5-2 mg) and place it on a tin disk previously cleaned with methanol. Fold the disc and analyze according to the procedure specified in section **Sample Analysis**. Analyze three cystine standards for each cast.

3. In CARIACO, two casts are done where 19 samples are taken for PC and PN analysis; therefore, six blanks and six standards are analyzed together with the samples, 25 in total, during each run on the analyzer. There are two casts per water column profile.

Calculation and Expression of Results

A calibrating factor for carbon (Kc) and nitrogen (Kn) is determined (in terms of µg C and N) using the following formula:

$$\text{K Factor}_{(\text{C or N})} = \frac{(A - B)}{(C * D)} \qquad (1)$$

where

A = sample counts.
B = blank counts.
C = sample weight.
D = standard theoretical weight (%).

With each K factor, the values for carbon and nitrogen in the sample are determined using the following equation:

$$\mu g\ L^{-1}{}_{(\text{C or N})} = \frac{(A - B)}{(\text{K Factor}_{(\text{C or N})} * V)} \qquad (2)$$

where

V = volume of filtered water, in liters.

Method 12

DETERMINATION OF NUTRIENTS USING CONTINUOUS FLOW ANALYSIS (CFA)

Kent Fanning, Robert Masserini and Laura Lorenzoni

Introduction

The analytical methods described follow the methodology of Louis I. Gordon, Joe C. Jennings, Jr., Andrew A. Ross and James M. Krest – "A Suggested Protocol for Continuous Flow Automated Analysis of Seawater Nutrients" (from WOCE Hydrographic Program and Joint Global Ocean Fluxes Study, Gordon *et al*., 2000) revised by Andrew Ross in August 2000 and modified afterwards by Kent Fanning and Robert Masserini from University of South Florida Nutrient Analysis Laboratory. Methods and flow diagrams of the configurations presented here are adequate to assess nutrients such as phosphate, ammonium, nitrate, nitrite and reactive silicic acid.

Principle of Analysis

Continuous flow analysis (CFA) is an automated method for nutrients analysis in which a Technicon Autoanalyzer II equipped with a multi-channel peristaltic pump is employed. The CFA reduces the technical error since it analyses the samples and the standard solutions in a similar way, improving precision when adding reagents. The introduction of air bubbles in the sample stream segments the flow, reducing mixing of adjacent samples and enhancing mixing of the reagents. Each chemical reaction takes place within a glass spiral coil (manifold) where mixing occurs. The sample-reagent mixture reacts chemically to produce a colored compound whose light absorbance is proportional to the concentration of the species being analyzed. A photometer located at the end of the CFA system measures color absorbance.

Analytical Warnings

1. Temperature fluctuations in the laboratory can cause errors in the kinetic of the reaction that generates color in the sample. This problem is evident in silicic acid analysis. Very high temperatures lead to degassing of fluid, causing interference in the sample stream segments.
2. Nutrients samples continue degrading after freezing. Analyze as soon as possible. Do not freeze silicic acid samples; instead keep samples refrigerated.
3. Suspended matter can interfere with the result so filter the sample before storing.
4. Deionized water (DIW) is not adequate for the baseline of the system or as a "wash" between samples and standard solutions. DIW is only recommended to establish the analytical zero. Seawater or artificial seawater with low nutrient concentrations, with salinities between the ranges of salinity of the samples, must be used in order to avoid introducing noise during the analysis or alter the refraction index.
5. Maintain constant hydraulic pressure along the system in order to avoid false signals.
6. Calibrate gravimetrically at a constant temperature

any plastic (polypropylene, etc.) labware used when preparing standard solutions, since temperature changes affect internal volume.

7. Exclude bubbles from the Cadmium-Copper column (Cd-Cu column).

Field Materials

1. Membrane filters (Nucleopore Track-Etch, 47 mm, or equivalent).
2. Filtration unit. Polycarbonate filter folders should be used to filter samples. Clean these by submerging in 5% HCL for 1-2 hours and rinsing several times with distilled water.
3. Vacuum pump.
4. Polyethylene bottles (250 mL) and high density polyethylene bottles (HDPE, 30 mL). Clean by submerging in 10% HCl all night and rinsing several times with distilled water

Laboratory Materials

1. Glass bottles calibrated gravimetrically (NIST quality Class A or equivalent).
2. Pipettes (Eppendorf Maxipettor, Lab Industries Standard REPIPET, and precision 0.1%).

Equipment

1. Technicon Autoanalizer II (AA-II) or an Astoria-Pacific International analyzer (former Alpkem) series 300 or RFA-2.
2. Peristaltic pump.
3. Analytical package including injection system, mixing coils, and water bath.
4. System structure. Follow Gordon *et al.* (2000) specifications (Figure 12.1).

Sampling

1. The interior of the Niskin bottles and the valves must be free of any inorganic or organic residue, as well as of any sign of corrosion. Replace any oxidized component and clean the interior of the bottles with 8M HCl.
2. Samples are collected in 250 mL polyethylene bottles. Rinse three times with the sample. Fill the bottle about ¾ full; try not to touch the cap or the bottle mouth with the fingers since fingerprints contaminate the sample with phosphates.
3. Filter the sample through a Nucleopore filter directly into a HDPE bottle, after rinsing three times with filtered water. Change the filter every four samples.
4. Fill the bottle to ¾ its volume, wipe the cap and the mouth of the bottle with a paper towel that does not release fibers and replace the cap firmly. Freeze immediately to temperatures below -20 °C. Keep the bottles vertically so they do not turn over and loose the brine. During the freezing process a brine forms which can be lost if the cap is not firmly tight. Lost of this brine can produce important errors.
5. Before the analysis, defrost the samples for a period of 15 minutes using a water bath. Mix before opening the bottle to guarantee a homogenous sample.
6. Silicic acid samples are filtered but not frozen. Store at 4 °C keeping the bottles vertically in a rack so they do not over-turn.

Preparation of Deionized Water, Low Nutrient Seawater, and Standard Solution

1. *Deionized water (DIW) and Low Nutrient Seawater (LNSW)*

Follow Gordon *et al.* (2000) specifications for DIW and LNSW used for reagents, standard preparation and analytical zero.

2. *Preparation of Standard Solutions*

The calculations of molar concentrations used here come from compiled tables from Weast *et al.* (1985, pp. D-232 y D-262). Write down the date and hour of standard preparation as well as the date of initial use.

Standard solutions used during the analysis are of three types: A, B and C.

- ***Standard A.*** Stock solution from which standard solutions B and C are prepared. Therefore, it is fundamental to use analytical grade reagents for standard A.
- ***Standard B.*** Standard solution prepared from the stock solution.
- ***Standard C.*** Working solutions introduced directly into the CFA for calibration (prepared with LNSW and standard B). Three standard C solutions with different concentrations are prepared.

Prepare two standards A and measure both in the AA-II. The maximum absorbance of both standard solutions must be identical; otherwise, prepare a third standard A and continue until two solutions have the same maximum absorbance.

Ground carefully analytical reagents in crystal form in a mortar. Dry each reagent in an oven to 105 °C for 48 hours and place in a vacuum desiccator with BaO or $MgSO_4$ for another 48 hours. Allow the reagents reach room temperature before weighing.

a. ***Standard A for phosphate and nitrate solutions (2,500 µM HPO_4^{2-} and 37,500 µM NO_3^-).*** Weigh 0.3402 g anhydrous potassium dihydrogen phosphate (KH_2PO_4; Fisher Scientific no. P382-500) and 3.7912 g potassium nitrate (KNO_3; Fisher Scientific no. P383-500). Transfer to two 1000 mL volumetric flasks (one for each reagent). Dissolve solids in DIW and dilute to 1 L.

b. ***Standard A nitrite solution (2,000 µM NO_2^-).*** Weigh 0.1380 g of sodium nitrite ($NaNO_2$; Fisher Scientific no. S347-500) with a 97-98% degree of purity and transfer to a 1000 mL volumetric flask. Dissolve in DIW and dilute to 1 L.

c. ***Standard A ammonium solution*** **(30,000 µM NH_4^+)**. Weigh 1.6037 g of ammonium chloride (NH_4Cl, 99% purity, Sigma Aldrich no. 32,637-2) and transfer to a 1000 mL volumetric flask. Dissolve in DIW and dilute to 1 L.

d. ***Standard A silicic acid (20,000 µM $SiOH_4$)*:**

i. Transfer 2 g of SiO_2 analytical grade to a platinum crucible and place in a burner for 5 minutes. Place the crucible in a desiccator and let it reach room temperature.

ii. Weigh 0.6008 g of dry SiO_2 and place in the platinum crucible.

iii. Weigh separately 4 g of sodium carbonate (Na_2CO_3) and place on the SiO_2 (Figure 12.2A).

iv. Cover the crucible and place on the burner in an angle (Figure 12.2B).

v. Keep away from the blue flame and heat until a cracking sound occurs. Remove from the burner. Replace the crucible on the burner and

heat until you hear the sound again. Repeat these steps until the sound stops. At this point, heat for 20 more minutes.

vi. Remove from the burner and let the crucible cool down.

vii. Transfer (with a spatula and DIW) the melted silica disk to a Teflon® beaker. Allow the disk to dissolve (about 30-45 minutes).

viii. Transfer the silica solution to a 500 mL volumetric flask and dilute with DIW. Work fast in order to avoid contamination from the glass. If more than 500 mL is needed, prepare two solutions of 500 mL and mix.

ix. Adjust the pH of the solution to 9.5 using 10% HCl.

x. Keep in a polyethylene bottle (never use glass) at room temperature. Never refrigerate since precipitation may occur due to the change of temperature.

e. ***Standard B for phosphate and nitrate (75 µM HPO_4^{2-} y 375 µM NO_3^-).*** Transfer 30 mL of phosphate standard A and 10 mL of nitrate standard A to a 1000 mL volumetric flask. Dilute to 1 L with DIW.

f. ***Standard B silicic acid (2,000 µM $SiOH_4$).*** Transfer 100 mL of standard A silicic acid to a 1000 mL volumetric flask. Dilute to 1 L with DIW.

g. *IT IS NOT NECESSARY TO PREPARE A STANDARD B FOR AMMONIUM AND NITRITE, SINCE STANDARD A FOR BOTH IS DILUTED ENOUGH.*

h. ***Working standard C solutions.*** Prepare three different concentrations of standard C for each nutrient to be analyzed (C1–C4, being C4 the highest concentration and C1 the LNSW blank). Table 12.1 indicates the volume from standard B (or A in case of ammonium and nitrite) that must be transferred to a 250 mL volumetric flask to make working solutions C2-C4. Dilute standard solutions A and B with LNSW in different proportions to make working solutions. The dilutions given in this manual are adequate for upwelling waters where anoxia is present. For other systems, adjust the dilutions according to the nutrient concentration range.

i. ***Surfactant agents.*** High purity dodecyl sodium sulfate ($CH_3(CH_2)_{11}OSO_3Na$) is used for the silicic acid and phosphate method, and Brij-35 is used for nitrate-nitrite analysis.

Nutrient Analysis

The following section gives details about the methods employed for nutrient analysis when using an AA-II. Maintain a regular pattern of bubbles, necessary for noiseless signals, by cleaning the flow channels daily with a solution of 1.2M HCl (10% HCl). Use DIW to rinse all tubes. Occasionally, wash with 2.5 M NaOH. Purge the reagents out of the system, so that they do not precipitate with the acids or bases used for washing. If the pattern of bubbles is interrupted, wash all the system and do not reinitiate immediately. The peristaltic pump and the air bar generate the segmented flow.

Phosphate

This procedure describes the method for the determination of phosphate in seawater using a modification of the procedure of Bernhardt and Wilhelms (1967). The results are expressed in µM of HPO_4^{2-}. The flow diagrams for the AA-II are in Figure 12.3. For this system, flow cells of 5 cm and S1 phototubes of high

rank are used. This method has a linear response of up to 5.0 µM HPO_4^{2-}.

Principles of Analysis

The dissolved orthophosphate ions (H_2PO_4, HPO_4^{2-} and PO_4^{3-}) in seawater react with the ammonium molybdate under acid conditions to form a complex compound (molybdophosphoric acid). The resulting complex is reduced when hydrazine sulphate is added forming a solution of an intense blue color. The flow of the sample in the autoanalyzer is heated to accelerate the reaction.

Reagents

Follow Gordon *et al.* (2000) instructions for the preparation of reagents.

Nitrate + nitrite

This procedure describes the method for nitrate + nitrite determination in seawater using a modification of Armstrong *et al.* (1967). The results are expressed in µM of NO_3^- + NO_2^-. The flow charts for this analysis are in Figure 12.4.

Principles of Analysis

The nitrate present in the sample is almost quantitatively (> 95%) reduced to nitrite ions when the samples pass through a Cd-Cu column. These nitrite ions represent the reduced nitrate plus any original nitrite ions. These ions react in an acid solution with two aromatic amines (sulfanilamide and N-(1-naftil)-ethylendiamine dihydrochloride) to form a reddish colored azo dye. The difference between the nitrite concentration in a reduced sample and the concentration in a non-reduced sample represents the concentration of nitrate.

Reagents

Follow Gordon *et al.* (2000) instructions for the preparation of reagents and Cd-Cu column. Figure 12.5 shows the Cd-Cu column appropriate for an AA-II where:

1 = Teflón® tube (internal diameter 1mm)
2 = Tygon® tubing
3 = connection Technicon N-6
4 = Tygon® tubing (1/4")
5 = glass wool cork
6 = Cd-Cu filings
7 = glass tube (external diameter ~1/4", 3" length)

Nitrite

The analysis is performed in a separate channel using the method for nitrate + nitrite but without neither the reduction column nor the Imidazole solution. Figure 12.6 shows the flow chart of the nitrite system. The reagents for this analysis are the same described for the nitrate analysis. The sensitivity of this analysis is approximately 0.056 AU/µM. The results are expressed in µM of NO_2^-.

Silicic acid

This analisys describes the method for silicic acid determination in seawater using the procedure of Armstrong *et al.* (1967) adapted by Atlas *et al.* (1971). Figure 12.7 shows the flow chart for the silicic acid system. This method is accurate enough to measure silicic acid in oceanic water (blue waters). The results are expressed in µM of $Si(OH)_4$.

Principle of Analysis

When adding molybdic acid to the sample, a complex named ß-silicomolybdic acid is formed, due to the reaction between ammonium molybdate in an acid solution and the silicic acid that the sample contains. When adding stannous chloride this complex is reduced to a blue color. The color development is sensitive to temperature fluctuations; therefore, the manifold should be protected from air drafts, and the temperature in the manifold should be controlled during the analysis (Knap *et al.*, 1997).

Reagents

Follow Gordon *et al.* (2000) instructions for the preparation of reagents.

Ammonia

From Gordon *et al.* (2000), the ammonia determination as described is modified from a method ALPKEM FRG, taken from "Methods for Chemical Analysis of Water and Wastewater", March 1984, EPA-600/4-79-020, "Nitrogen Ammonia" , Method 350,1 (Colorimetric, Automated Phenate). The flow chart for this method is in Figure 12.8. The AA-II uses a flow cell of 50 mm, interference filters of 640 nm and Technicon S-10 phototubes. This method is linear at least until 6 µM. Analytical sensitivity is 0.022 AU/µM ammonium.

Principle of Analysis

The ammonium in the sample reacts with alkaline phenol in the presence of sodium hypochlorite as an oxidizing agent to form the complex blue indophenol. Due to the high pH, a complexing agent is used to remove the excess of magnesium ions that form during the reaction and interfere in the analysis.

Reagents

Follow Gordon *et al.* (2000) instructions for the preparation of reagents.

Sample Analysis

A summary of the steps to follow in order to run seawater samples through the CFA system is given below.

1. ***Start the equipment and establish the baseline***
   a. Check the levels of DIW and "wash" solutions (DIW and surfactants) in the bottles and fill as needed.
   b. Start pumping and purge the system at least for 10 minutes. The longer the purge, the better the run will be.
   c. While purging, check the volume of all the reagents and fill as needed. Prepare new working solutions.
   d. Assure a constant airflow and the presence of bubbles in the flow system. This guarantees a suitable purge of the air from the system.
   e. Once the purge is finalized, check the voltage scales (zero and maximum, 5V FS) in the equipment and adjust them as needed.
   f. Add the reagents by moving the reagent tubes from the washing bottles to the corresponding reagent bottles.

      NOTE. In the silicic acid channel, it is necessary to wait at least 5 minutes before adding the $SnCl_2$ after the addition of the tartaric acid and molybdate solutions. A premature addition of the $SnCl_2$ can lead to the formation of a dark blue precipitate on the walls of the tubes and mix-

ing coils. If this happens, purge the system with NaOH to dissolve and remove the precipitate.

g. It is important to assure the purge of all the system with buffer solution (Imidazole) before introducing the Cd-Cu column. Usually this solution runs throughout the system for 3 to 5 minutes after the pumping begins.

h. Changes in the baseline of the detector are observed when the reagents reach the colorimeters. At this point, write down the difference (in graph units) between the original baseline, using DIW, and the new baseline using the reagents. This difference must be consistent day after day, and is a good mechanism to confirm the correct operation of the system (the opposite indicates deterioration of the reagents or a change in the calibration of the colorimeters).

i. Allow the instrument to register the new baseline for 10-20 graph units until obtaining a stable baseline where irregular changes can be observed. If the baseline has noise, check that there are not bubbles clogged within the system. Usually pinching the tube at the exit flow of the cell within the colorimeter for several seconds solves the problem.

j. If the baseline does not display noise, start running the samples. Place the washing tube (that fills the autosampler) in the LNSW. Observe the detector and wait for the change of signal that indicates that the LNSW is flowing through the system and has reached the colorimeters (around 20 minutes)

NOTE. It is important to label properly the bottles corresponding to each working standard and to the DIW, to avoid confusion at the time of the run.

2. ***Sample run***

a. Run working standards before and between samples. The run of standards and samples consist of:

- A run of the working standard C4.
- Two runs of the standard C1 (LNSW).
- Three runs of the working standards C4.
- Three runs of the working standards C3.
- Three runs of the working standards C2.
- Three runs of the standard C1 (LNSW).
- Three wash with DIW.
- Run of the first group of seawater samples, running the first sample two times.
- After 12 samples, run the baseline with LNSW, to verify that the drift of the equipment is less than two graph units.
- Run the rest of the samples, repeating the baseline of LNSW after every 12 samples (if it is necessary).
- A run of LNSW.
- Three wash with DIW.
- Three runs with standard C1 (LNSW).
- Three runs with the working standard C2.
- Three runs with the working standard C3.
- Three runs with the working standard C4.
- Allow the baseline to run for 5 minutes.

NOTA. A run of more than 12 samples usually takes several hours. There is a delay between the time at which the sample is introduced to the system and the time it takes the colorimeter to detect a peak (for ammonium, the lag time can be of up to 20 minutes). Allow the run of the baseline for 5 minutes before initiating a second run or culminating the present one.

During the run, air bubbles must have a regular pattern. Check for noise or sudden changes in the measurements of the colorimeter in the detector, as well as in the extractor tube. The extractor tube must take the sample correctly from each bottle, and care should be taken to avoid wrong positioning or movement of the bottles on the carousel, which can affect the procedure. Verify the levels of the reagents during a run. In order to avoid the drift of the instruments, the runs for a set of samples must be brief, so that the time that passes between the beginning and the end does not exceed 90 minutes.

3. ***Purging and turning off the system***

a. Once the baseline has run for 5 minutes at the end of a series of measurements, it is possible to begin to wash the system and turn it off. The "wash" is fundamental to avoid the formation of precipitates in the walls of the tubes and mixing coils of the system.

b. Turn off the detector and close the data acquisition program.

c. Carefully remove the Cd-Cu column from the system, trying not to introduce air within the column while doing it.

d. Remove all the reagents, except tartaric acid, and place all the tubes in their respective "wash" recipients. Once the $SnCl_2$ is purged completely from the system, place the tube of tartaric acid in its "wash" recipient.

e. Using the autosampler, run two bottles with 10% HCl, two bottles with NaOH 1M and two bottles with 10% HCl through all the system.

f. Purge the system with DIW for about 10-15 minutes before turning off the pump.

Calculation and Expression of Results

For calculations and expression of results, see Gordon *et al.* (2000).

Table 12.1.
Volume of standard B (or A in case of ammonia and nitrite) to prepare standard solutions C. LNSW is low nutrient seawater.

Nutrients Standard solutions	Phosphate	Nitrate	Silicic acid	Nitrite	Ammonia
C4	15 mL	15 mL	10 mL	0.25 mL	0.25 mL
C3	10 mL	10 mL	5 mL	0.1 mL	0.1 mL
C2	5 mL	5 mL	2.5 mL	0.05 mL	0.05 mL
C1	**LNSW**	**LNSW**	**LNSW**	**LNSW**	**LNSW**

Method 13

DETERMINATION OF CHLOROPHYLL *a* AND PHAEOPIGMENTS

Ramón Varela

Introduction

The determination of chlorophyll *a* is used as an indicator of the biomass of the primary producers (phytoplankton) that are found as suspended particles in the water. The following procedure quantifies the chlorophyll concentration and phaeopigments found in a sample using fluorometric techniques. The procedure follows Holm-Hansen *et al.* (1965), and the calculations are done as indicated in Lorenzen (1966). Among the modifications are the use of methanol instead of acetone as an extraction solvent due to its greater efficiency (Holm-Hansen and Riemann, 1978) and the use of a sonic dismembrator (Wright *et al.*, 1997). The method applies to all ranges of chlorophyll *a* concentration found in seawater. The method detection limit is 0.01 µg L^{-1} for natural waters (for a 0.5 L sample). The results are expressed in µg L^{-1} or mg m^{-3}.

Principle of Analysis

A seawater sample is filtered through a fiberglass filter on which suspended particles are retained. Among these particles, there is chlorophyll bearing organisms. Algal pigments are extracted using methanol as the solvent and a sonic dismembrator is used to break the filter and the cells. After clarifying the extract by centrifugation, algal pigments are excited by light, which has a blue wavelength, emitting fluorescence with a red wavelength. The fluorescence is detected by a photomultiplier. Next, the sample is acidified to convert all the chlorophyll *a* into phaeopigments and is measured again in the fluorometer.

Analytical Warnings

1. The presence of chlorophyll *b*, chlorophyll *c* and/or divinyl chlorophyll *a* in the sample can cause error in the measurement. High chlorophyll *b* concentrations, especially at the depth of the chlorophyll maximum, have a negative interference with chlorophyll *a*, and a positive one with phaeopigments (Knap *et al.*, 1997). This interference is important if algae of the class Chlorophyceae and Prochlorophyceae are abundant.
2. The detection limit of the equipment is exceeded when the level of pigments in the extract is very high. The fluorometer is always kept at maximum sensitivity; therefore, the measurements tend to underestimate high concentrations, possibly because of quenching. In order to solve this problem, several alternatives exist. One alternative, not recommended, is to dilute the sample, which increases the uncertainty of the method. If the filter is saturated after filtration, one option is to cut the filter into smaller fractions and analyze separately. The most recommended option in very productive waters with high concentration of phytoplankton is to filter a smaller volume of water (250 mL or less) to avoid cutting the filter or diluting the sample.
3. Fluorescence depends on temperature; therefore,

perform the calibration of the equipment as well as analysis of samples at a constant ambient temperature between 20-25 °C. The temperature coefficient for the fluorescence of chlorophyll is - 0.3%/°C.

4. Chlorophyll degrades quickly under intense light; perform the analysis under dimmed light.
5. Materials used during the analysis as well as the methanol must remain free of acid residues.

Materials

1. Fiberglass filters (Whatman grade GF/F or equivalent, 0.7 µm pore size, 25 mm diameter).
2. Funnels for 25 mm filters, 200 mL capacity.
3. Polypropylene graduated centrifuge tubes with screw caps for extraction, 15 mL.
4. Cells, 13-mm, specific for the fluorometer.
5. Automatic pipettes with capacity of 200 and 1000 µL.
6. Volumetric pipettes (5 and 10 mL).
7. Small spatulas.
8. Vinyl gloves.

Equipment

1. Fluorometer Turner Designs (10-AU-005), with a photomultiplier sensitive to the red wavelength (λ 185-870 nm), lamp F4T5/d, blue filter (λ 340–500 nm CS 5-60) and red filter (λ > 665 nm CS 2-64).
2. Sonic dismembrator, Fisher Scientific model 100.
3. Centrifuge, 0 - 3900 r.p.m.
4. Filtration unit and vacuum pump.

Reagents

1. ***Methanol*** (CH_3OH).
2. ***Hydrochloric acid*** (0.48N HCl).
3. ***Concentrated chlorophyll a solution***. An ampoule of commercially available pure chlorophyll *a* standard solution is used (*Anacystis nidulans* C-6144 Sigma, 1 mg) free of chlorophyll *b*. Dissolve the content of an ampoule filled with chlorophyll *a* crystals in 15 mL of methanol. Chlorophyll crystals dissolve very slowly; therefore, make the dilution two days before the calibration. Once dissolved, keep this solution frozen at - 20 °C.
4. ***Working solution for calibration***. Dilute 1 mL of the concentrated chlorophyll *a* solution in 49 mL of methanol. The concentration of this solution must be approximately 1 µg L^{-1}. In order to determine the precise concentration, measure the absorption for each nanometer between 660 and 670 nm using a recently calibrated spectrophotometer and locate the wavelength of the maximum absorption (A_{max}). Determine the chlorophyll *a* concentration in the solution applying the following formula:

$$\text{Chlorophyll } a = \left[\frac{(A_{max} - A_{750nm})}{E * L} \right] * \frac{1000 \text{ mg}}{1 \text{g}} \qquad (1)$$

where

A_{max} = maximum absorption (between 660 - 670 nm).

A_{750nm} = absorbance at 750 nm.

E = chlorophyll specific absorption coefficient in methanol, 79.81 g^{-1} cm^{-1} (Jeffrey and Welschmeyer, 1997).

L = cell pathlength in cm, 10-cm.

Cleaning Procedures

Soak all the glass material (cells, pipettes, etc.) in a 10% HCl solution for 24 hours. Rinse five times with

distilled water and let them dry. Keep the fluorometer cells upside down to avoid dust particles inside the cell. Wash the tweezers, spatulas, and any other material that is not made of glass with liquid detergent, running water and a final rinse with distilled water. Wear gloves all the time.

Sampling

1. Collect the water samples in 1 L polyethylene dark bottles rinsing three times with the sample before filling.
2. Filter the sample immediately through a fiberglass filter. Register the filtered volume. The volume to filter depends on the amount of chlorophyll that is found in the water, which can be estimated through the CTD fluorometer. In waters with low biomass (< 1 mg m^{-3}), filter a volume of 500 mL in duplicate. If the biomass is > 1 mg m^{-3}, filter 250 mL in triplicate.
3. Maintain vacuum levels between 400-500 mm Hg to avoid breaking the cells.
4. When filtration is finished, remove the filter with a flat tweezers and fold in half or roll up with the side that contains particles inwards. Place in a centrifuge tube properly identified.
5. Freeze at -20 °C. Analyze within a month.

Blank Analysis

Before beginning the chlorophyll *a* analysis, it is essential to evaluate the operation of the fluorometer as well as the analytical quality of the methanol with a blank analysis. The methanol must be free of polluting agents or of some other contaminant that can alter the results.

1. Place 5 mL of methanol with a clean pipette in a fluorometer cell.
2. Make a reading in the fluorometer. An indication of possible contamination of the reagent or uncalibrated equipment is a reading greater than zero; therefore, **DO NOT** make the analysis until the equipment is calibrated or after changing the reagent.
3. It is convenient to use a solid standard provided by the manufacturer to verify the stability of the equipment before the analysis. This standard is adjusted during the calibration.

Sample Analysis

It is recommended to begin the analysis with samples with lower concentrations and finish with the ones with greater concentration (usually surface samples). Analyze duplicates simultaneously. Wear gloves all the time during the analysis and follow safety procedures for handling methanol.

1. ***Sample preparation before the analysis. Sonication*.**
   a. Remove the centrifugue tubes from the freezer, and let the filters reach room temperature protected from light.
   b. Filters are extracted in 10 mL of pure methanol. Verify that all the tubes contain the same amount of liquid and that the filters are totally submerged.
   c. Grind each filter within its tube with a small spatula to homogenize the sample. This procedure enhances the rupture of the algal cells and increases extraction efficiency of the solvent. Put the tubes in a test tube rack and cover with aluminum foil to protect the tubes from light.
   d. Lower the sonic dismembrator into the methanol of each tube without touching the bottom and the walls of the tube. The probe must be as perpendicular as possible.

e. Turn on the dismembrator and gradually increase the power until position 15.
f. Sonicate for 30 seconds, turn off the equipment and remove the dismembrator, put the centrifuge tube cap on, and store the sample protected from light.
g. Rinse the tip of the dismembrator with pure methanol between samples and let it dry.
h. Place the tubes in a rack and store at 4 °C (do not freeze) for 24 hours to allow methanol to extract the pigments. It is recommended to shake slightly the tubes three or four times during this period.

2. ***Measurement of chlorophyll a and phaeopigments with a fluorometer***

Turn on the fluorometer one hour before beginning the analysis to allow the equipment to stabilize.

a. Place the tubes in a centrifuge for 30 min at 3000 r.p.m. to clarify the extract.
b. Transfer the tubes to a rack and arrange duplicates together, protecting them from light.
c. With a pipette, carefully withdraw 5 mL of methanol from the upper portion of the liquid in the tube, without touching particles of the filter or producing turbulence. Add to a fluorometer cell.
d. Place the cell in a previously calibrated fluorometer, and make the reading once the equipment stabilizes (~ 8 s). This reading corresponds to the initial fluorescence (Fo).
e. Add 100 µL of 0.48N HCl and shake slightly. Wait at least three minutes to complete the reaction.
f. Place the cell in the fluorometer and once it stabilizes, make the reading of the acidified sample. This reading corresponds to the acidified fluorescence (Fa).
g. Between samples, rinse the pipette and the fluorometer cells a minimum of three times with pure methanol.

Fluorometer Calibration

Calibrate the fluorometer every six months with a pure, commercially available chlorophyll a standard (*Anacystis nidulans* C-6144 Sigma, 1 mg) free of chlorophyll b. Follow the instructions of the manufacturer. Make five dilutions of the working solution of chlorophyll, with concentrations between 0 and 130 µg L^{-1}, which allows calibrating the equipment for a scale of 0 to 150 µg L^{-1}. Once calibration is finalized, measure the solid standard that is provided by the manufacturer, and use this reading to verify the stability of the equipment in successive analyses. A drift greater than 10% indicates the necessity of a new calibration.

The same dilutions can be used to determine the acidification coefficient (R) after calibration. Take the readings from several dilutions (Fo_{max}), then acidify with 100 µL of 0.48N HCl. Measure samples again in the fluorometer (Fa_{max}). R is the maximum fluorescence ratio, before and after acidifying, calculated from the chlorophyll standard a (pure) used to calibrate the fluorometer. For methanol, R varies between 2.4 and 2.7. The value of R for methanol is more variable that the one used for acetone. The amount of acid added to the cell must be the same for the calibration as well as for the samples. A small increase in the volume produces anomalous values of R.

$$R = \frac{Fo_{max}}{Fa_{max}} \qquad (2)$$

where

Fo_{max} = reading before acidification.
Fa_{max} = reading after acidification.

Calculation and Expression of Results

The Lorenzen formula (1966) used to calculate the chlorophyll *a* concentration and phaeopigments in seawater is:

$$\text{Chlorophyll } a \text{ } (\mu g\ L^{-1}) = \left[\frac{R}{(R-1)}\right] * (Fo - Fa) * \left(\frac{Ve}{Vm}\right) \quad (3)$$

$$\text{Phaeopigments } (\mu g\ L^{-1}) = \left[\frac{R}{(R-1)}\right] * ((R * Fa) - Fo) * \left(\frac{Ve}{Vm}\right) \quad (4)$$

where

Vm = volume of methanol (10 mL).
Vs = volume of filtered sample.
Fo = reading before acidification.
Fa = reading after acidification.

Method 14

DETERMINATION OF PRIMARY PRODUCTION

Ramón Varela

Introduction

The primary production rate is defined as the amount of inorganic carbon assimilated by phytoplankton during photosynthesis that is converted into particulate organic matter per unit of time and volume (mg C m^{-3} h^{-1}), or surface area (g C m^{-2} d^{-1}). This last definition is obtained when integrating a series of production measurements over different depths. The production rate can be measured using radioactive carbon-14 (^{14}C) labeled sodium bicarbonate following the method of Steeman-Nielsen (1952) with changes indicated by Knap *et al.* (1997), IOC (1994), Strickland and Parsons (1972) and Ros (1979). This method allows detection of carbon fixation rates between 0.05 and 100 mg C m^{-3} h^{-1}. The procedure that is described fits the conditions of high productivity that are found in coastal waters at the CARIACO station. Specifically, the variables that have been modified are the level of specific activity of the sodium (^{14}C)-bicarbonate solution, the time of incubation at sea and the volume of water that is filtered after the incubation.

Principle of Analysis

Assimilation of dissolved inorganic carbon (DIC) by phytoplankton is estimated when a small amount of ^{14}C is added in the form of $NaH^{14}CO_3$ to a water sample and allowing the phytoplankton to incorporate the tracer in the organic matter during photosynthesis. After adding ^{14}C, the samples are incubated "*in situ*" at the depths of origin of each sample for a minimum period of 4 hours. If the total content of CO_2 concentration in the water sample is known as well as the amount of ^{14}C added, the primary production rate can be calculated.

Analytical Warnings

1. High levels of light and changes in temperature can alter the results. Phytoplankton from deeper layers are adapted to low levels of light; therefore, when these organisms are exposed to brief periods of intense light, the photosynthesis process may speed up or a light or thermal shock may occur. It is important to perform sampling, handling, and filtration of the samples under conditions of dimmed light and at a stable temperature similar to sea surface temperature.
2. Contamination with trace metals such as Cu, Zn and Fe inhibits carbon fixation. Avoid the contamination by following recommended techniques for cleaning the material, including the Niskin bottles. A suitable cleaning of the instruments and containers used during the analysis assures data quality.
3. Apply a strict protocol with the material and instruments that make contact with the solution of ^{14}C to avoid contamination from the radioisotope during manipulation as in the work area. Special care with the pipettes is important and avoids transferring the radioisotope between samples. Use trays and absorbent paper for containing spills, and follow safety techniques recommended for this kind of work.
4. CO_2 can be easily lost to the atmosphere from seawater samples; therefore, aliquots taken from

the incubation vessels to measure radioactivity are preserved in vials containing 200 µL of 2-phenethylamine (a CO_2 absorber).

Materials

Use only HCl and distilled water to wash the materials that will be in contact with the sample. Do not use detergents or disinfectant cleaners as this may affect the activity of the organisms. Wear polyethylene or vinyl gloves all the time for handling the materials and the preparation of reagents, to avoid contaminating samples and for safety.

1. Glass filtering flasks, capacity 2 L.
2. Polycarbonate funnels for 25 mm diameter filters, capacity 250 mL.
3. Fiber-glass filters Whatman GF/F, diameter 25 mm.
4. Automatic pipettes with capacity of 200 and 1000 µL.
5. Polycarbonate erlenmeyer flasks with screw caps, capacity 300 mL, for incubation (grouped in four for each depth plus a fifth as a blank).
6. Plastic syringes of 60 mL.
7. Glass scintillation vials, capacity 20 mL.
8. Polyethylene or vinyl gloves (powder-free).
9. Tray and absorbent paper, for catching spills in the filtration unit.
10. Buoy, ballast, and line with coastlock snap swivels (or equivalent) to hold flasks on the line (Figure 14.1).

Reagents

1. ***Sodium (^{14}C) bicarbonate working solution,*** **($NaH^{14}CO_3$)**. Approximate activity of 11 µCi mL^{-1}. Refer to Appendix 1 for the solution preparation.
2. ***Hydrochloric acid (HCl 0.5 N).***
3. ***2-phenethylamine*** ($C_6H_5CH_2CH_2NH_2$), Sigma-Aldrich, Inc., P-2641.
4. ***Scintillation liquid (CytoScint®),*** or similar compatible with aquatic samples.

Equipment

1. Filtration unit and vacuum pump.
2. BetaScout liquid scintillation and luminescence tester, PerkinElmer 2007-0010.

Sampling

Wear polyethylene or vinyl gloves all the time during sampling and sample analysis. See Figure 14.2 for a schematic detail

Hours before the cruise

1. Wash all the material used in the field with 1% HCl and distilled water. Avoid contact with metallic objects. If the erlenmeyer flasks are new, soak them in 5% HCl for 72 hours. Rinse three times with deionized water. Store the flasks filled with 100 mL of 1% HCl while they are not in use between cruises.
2. Wash the pipette tips with 5% HCl and rinse four times with distilled water. Dry them and store the tips in a polyethylene glove or in a Ziplock® bag until they are used.
3. Under a fume hood, add 200 µL of 2-phenethylamine to each scintillation vial that is going to hold water during the analysis. Mark these vials as **S**.
4. Calculate the exact volume of the working bicarbonate to add to each incubation flask, based

on its activity, which must be measured previously with the scintillation tester (i in **NOTE 1**).

In the field

1. One or two hours before dawn obtain the samples in order to have enough time to prepare the production array before sunlight is too intense (Figure 14.1). Collect five water samples from the Niskin bottle directly into polycarbonate flasks for each depth. Rinse three times with the sample, and fill leaving a small air space. Three of the flasks are going to be exposed to light (**L**), the fourth flask corresponds to the "dark bottle" (**D**), and the fifth is used for the measurement of the blanks (**B**) which is only required at some depths (Figure 14.2). In CARIACO, primary production is measured in eight depths: 1, 7, 15, 25, 35, 55, 75 and 100 m, which includes in this case the totality of the euphotic zone.
2. Keep filled flasks separated by depth in labeled boxes or black bags protected from light while sampling from the rosette.
3. In the laboratory of the boat and under dimmed light conditions, add the working bicarbonate solution (~ 11 µCi mL^{-1}) to all the flasks **L** and **D**. The volume that is added to each flask must provide an activity between 4 µCi.
4. Withdraw 200 µL of water from one flask marked **L** for each depth and transfer into a vial marked **S** (that contains 2-phenethylamine), label each vial according to the depth (**z**) of origin (**Sz**). This step is made to determine the radiation levels added to the flasks. Wrap each flask **D** with aluminum foil, as they represent the "dark bottles" which measure dark carbon fixation for each depth.
5. Withdraw 200 µL from each flask without ^{14}C (blanks, **B**) at each depth and transfer to vials marked as **So**. This measures the background natural radiation of seawater and particles.
6. Next, two 30 µL aliquots are taken from the working bicarbonate solution to determine the activity of the solution. Transfer to two vials marked with **S** and differentiate as **Sc**.
7. Withdraw 50 mL of seawater from flask **B** and filter to determine the blank. When finish filtering, rinse the filter with 250 µL of 0.5 N HCl, and place it unfolded into an empty vial (without 2-phenethylamine) with the side of the filter retaining particles upwards, and mark according to the depth as **Bz**.
8. If it is estimated that the production is low (< 1 mgC m^{-3} h^{-1}), increase the volume to filter to 150 mL for all the blanks as well as for all the samples. Record this volume.

***Incubation* in situ**

1. Incubation is done in the water using a production array. Attach a buoy to a line of 120 m length weighted with ballast, and marked with small swivels used to place groups of four flasks (3 **L**'s and 1 **D**) to the depths from which the samples were collected. This method of incubation allows that the samples remain exposed to the same temperature and level of natural light of the depth of origin (IOC, 1994). The buoy must be visible and easy to recover; therefore, it must be equipped with some location mechanism (flag, bright colors, reflecting radar, strobe flash, etc.).
2. Keep the flasks protected from light and in a cool place until deployment. Deploy the productivity array approximately at dawn. Write down time and position of deployment. Incubate the samples for a period of time that captures a representative portion of the luminance energy, which then is extrapolated

to the assimilation of a day. This depends on the luminance regime of the site (seasonally variable), as well as of the latitude. At the CARIACO station, the time of incubation is 4 hours at a minimum but no more than 5 hours for logistic reasons. The deployment starts between 6:30 and 7:00 A.M. and it ends between 10:30 and 11:00 A.M. This interval is also equivalent to 1/3 of the hours of light in a day and where 1/3 of the average daily solar energy to the station latitude is received (10.5° N, Figure 14.3).

3. Once the period of incubation is finished, recover the productivity array. Record the time and position of recovery. Detach each group of flasks from the line and place them in separate labeled dark boxes or bags, protected from light until filtration. Filter as soon as possible to avoid changes due to biological processes within the flask. It is important that the temperature of the sample do not reach 25 °C.
4. While keeping the laboratory under conditions of dimmed light, withdraw 50 mL aliquot from each flask (**L** and **D**) using a 60 mL plastic syringe. Filter into four individual funnels through 25 mm fiberglass filters maintaining vacuum levels of 500 mm Hg or less. Rinse the filter at the end with 250 µL of 0.5 N HCl. In periods of low biomass when low levels of productivity are expected, filter 100 mL or 150 mL instead of 50 mL. Place the filter in an empty scintillation vial, cover and refrigerate to 4°C until analysis can be completed. Identify each vial according to the depth of origin as **Lz** and **Dz**, conducting this operation in sequence from the shallower to the deeper sample.

Sample Analysis

In the laboratory

1. In the shore laboratory, add to all vials (**S**, **B**, **L** and **D**) 10 mL of scintillation liquid, slowly and without producing bubbles the same day that the samples are taken. Make sure that the surface of the filter is in complete contact with the liquid. Keep the vials protected from light and refrigerate until reading in a scintillation tester. Never freeze.
2. The vials with the liquid are measured in the scintillation tester after four weeks or when the filter looks almost invisible in the liquid. Do not shake the vials, verify that the filter is almost invisible, and the liquid is not turbid. Clean the vials externally with lens paper and make the measurement

Calculation and Expression of Results

The scintillation testers indicate the average number of light flash emissions or counts per minute (CPM) in vials that contain scintillation liquid. These counts are transformed into disintegrations per minute (DPM), since the equipment and the liquid do not have an efficiency of 100%, and not all the ß disintegrations are seen as an individual sparkle. Therefore, it is necessary to determine the efficiency of the equipment before running the samples using a well-known and stable DPM stock solution provided by the manufacturer of the equipment. This efficiency is used to correct the rest of the measurements. Once the values are corrected, production is calculated (in mg C m^{-3} h^{-1}) by depth using the following formula:

$$\text{Producción} = \frac{[\text{DPM(Lz)} - \text{DPM(Dz)}] * \left(\frac{V_{ws}}{V_{wf}}\right) * \text{DIC} * 1.05 * F_s}{[\text{DPM(S)}] * \left(\frac{V_{ws}}{V_{al}}\right) * 12} \quad (1)$$

where

DPM(**Lz**) = average DPM of the three filters in the transparent bottles (**L**) from a single depth corrected with the filter blank (**Bo**).

DPM(**Dz**) = DPM in the filter of the dark bottle (**D**) corrected by the filter blank (**Bo**).

V_{ws} = volume of water in the flask (290 mL, constant in this case).

V_{wf} = volume of filtered water in the flasks **L**, **D** and **B** (50, 100 or 150 mL).

DIC = concentration of inorganic carbon in seawater at sampled depths in mg C m^{-3}.

1.05 = constant describing the difference of diffusivity of the molecules with ^{12}C and ^{14}C in the live cells.

F_s = daily solar energy rate received during the incubation. Relative number calculated based in the area integrated under the solar radiation curve between the period of incubation for the latitude of the station (**NOTE 2**).

DPM(S) = DPM of the aliquots **Sz**, from the liquid extracted from the flasks **L** at the beginning of the experiment, or added radiation.

V_{al} = volume of the aliquot (or 0.20 mL) extracted from the flask to the vials (**Sz**)

12 = factor for the calculation of the concentration per hour. If the factor is ignored, the calculated production is per day.

NOTE 1. Preparation of the working solution of sodium bicarbonate ($NaH^{14}CO_3$), approximate concentration 11 µCi mL^{-1}

The amount of marked bicarbonate that is added to each flask must have an activity of 4 µCi, which is an adequate level for areas with high productivity (> 1 mgC m^{-2} d^{-1}) and short periods of incubation (4 hours). In order to obtain this activity, it is required to prepare an intermediate working solution, between the manufacturer's original activity (1 mCi mL^{-1}) and the final activity that is added to each flask (4 µCi). The radioactive isotope use is restricted under a series of regulations, safety measurements, and protocols for disposal of residues to the environment that varies according to the country. The investigators must know these details and follow their implementation. Perform all the activities related to this analysis on a tray to catch spills and on a table covered with absorbent paper with bottom plastic lining. Wear vinyl gloves all the time.

Materials

Wash all glass material with 10% HCl and distilled water, wrap in aluminum foil, and sterilize in a steam pressure sterilizer for 5 minutes. Remove humidity by placing in an oven to 70 °C for 3 to 4 hours.

1. Glass pipettes 5 mL.
2. Glass beakers, 100 and 500 mL.
3. Volumetric flasks, 100 mL.
4. Glass dropping bottle.

5. Nylon syringe filters, 25 mm diameter and 0.2 µm pore size.
6. Plastic syringes to filter water through the filters, 60 mL.
7. Tuberculin syringes with needle.
8. Serum tubing vials of 15 mL, with stoppers.

Reagents

1. ***Concentrated sodium (^{14}C) bicarbonate solution*** ($NaH^{14}CO_3$), specific activity 1 mCi mL^{-1}, Perkin Elmer, product number NEC086H001MC.
2. ***Distilled water*** coming from a glass still system.
3. ***Sodium hydroxide solution*** (**NaOH 0.1 N**).
4. ***Buffer solution*** with a pH 10
5 ***Hydrochloric acid*** (**HCl 0.1 N**).

Equipment

1. pH meter.
2. Magnetic stirrer.
3. Electronic balance, 0.01 g precision.
4. Steam pressure sterilizer

Preparation of the Solution

1. ***Distilled water pH adjustment***

   a. Add approximately 250 mL of distilled water to a beaker and cover.

   b. Calibrate the pH meter with a buffer solution of pH 10.

   c. Adjust the pH of the distilled water to pH 9.5 by adding, drop by drop, a solution of 0.1 N NaOH while mixing gently with a magnetic stirrer

2. ***Preparation of the bicarbonate solution (concentration 9-11 µCi mL^{-1})***

a. Tare a volumetric flask of 100 mL with its cap.

b. Using a plastic syringe of 60 mL with a nylon filter attached, add distilled water with adjusted pH to the flask placed on the balance until 60 mL has been added (determine the volume based on the weight aprox. 60 g).

c. With a tuberculin syringe, extract all the content of the ampoule that contains the concentrated bicarbonate solution and add it to the volumetric flask with the water.

d. Rinse the ampoule three or four times with filtered distilled water with adjusted pH, using the tuberculin syringe. Add the water used for rinsing in the volumetric flask that contains the bicarbonate solution.

e. Increase the volume in the flask to 90 mL (by weight, 90 g) with distilled water of adjusted pH using the 60 mL syringe with the filter.

f. Mix the content of the flask slightly.

g. Decant the working solution to 15 mL serum vials using 5 mL sterile pipettes. Fill six serum vials with 15 mL each. Seal with stoppers. Extract the air from the vial using a tuberculin syringe.

h. The specific activity of this working solution is 9-11 µCi mL^{-1}. Refrigerate at 4 °C by not more than six months.

i. The volume of the working solution of bicarbonate added to the incubation flasks to assure an activity of 4 µCi is calculated from the readings with the scintillation tester. This volume must be of 420 ± 45 µL. Before closing the last vial, extract 30 µL and add them to a scintillation vial

that contains of 200 µL 2-phenethylamine. Add scintillation liquid and measure in the scintillation tester the real activity of the solution and determine accurately the volume of solution necessary to obtain an activity 4 µCi by dose

$$\text{Activity in } \mu Ci\ mL^{-1} = (DPM(\mathbf{Sc}) / 2200000) / 0.03\ mL \quad (2)$$

The indicated volume assumes that each prepared vial will be used on a single cruise with a minimum residue. Thus, 15 mL will allow you to prepare 32 incubation flasks. The solution in the vials loses activity with time if they are not full due to CO_2 loss

NOTE 2. Fs calculation

For primary production calculations, a factor **F** can be used that takes into account not only the time for incubation, but also the solar proportion of the daily energy, which the samples receive. Because it is not possible to do the incubation in CARIACO for an entire daylight period (12 hours), it is done in ⅓ of this period. The amount of energy is not the same at the time incubation begins and that at the end. Using a sensor of luminance radiation (with cosine collector), the curve of daily solar radiation coming from the sky can be determined (Figure 14.3). These curves also can be calculated from tables based on the latitude and the month of the year. Solar factor **Fs** is the proportion of the area under the curve in the interval of the hours used for the incubation in relation to the total area under the curve. The object is to ensure the proportion is at least a third of the daily energy and that the number of hours is also a third of the period of light.

The previous statement can be ignored if the luminous energy is not taken into account but instead the hours of incubation are used, for which it is only necessary to eliminate the **Fs** factor in the numerator of formula (1), and replace constant 12 in the denominator by the time in hours of incubation in decimal fraction.

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FIGURAS / FIGURES

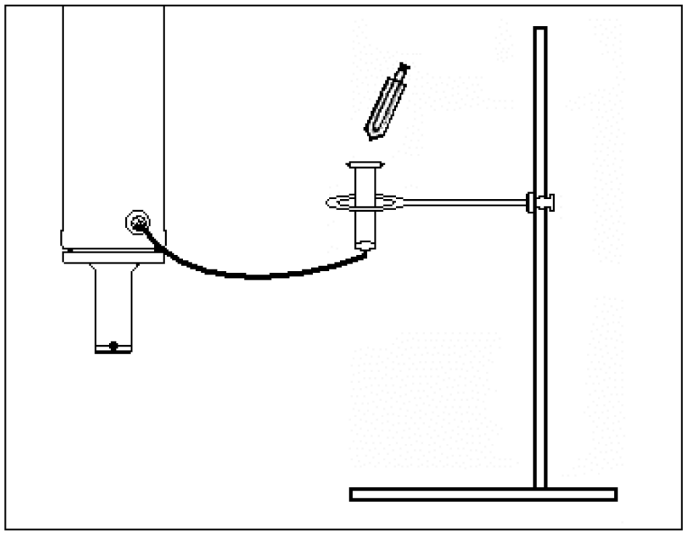

Figura 4.1
Método de captación de la muestra para H_2S.
Sampling method for H_2S samples.

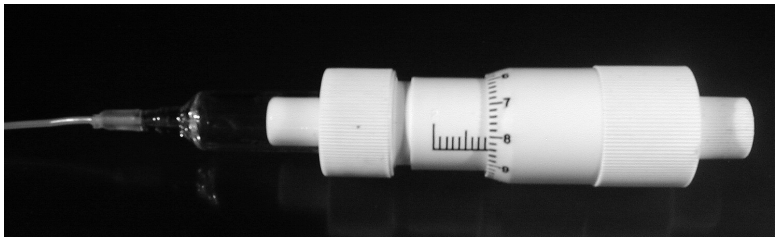

Figura 6.1
Jeringa micrométrica Gilmont para dispensar el indicador en los análisis de pH y alcalinidad.
Micrometric Gilmont syringe to dispense the indicator in the analysis of pH and alkalinity

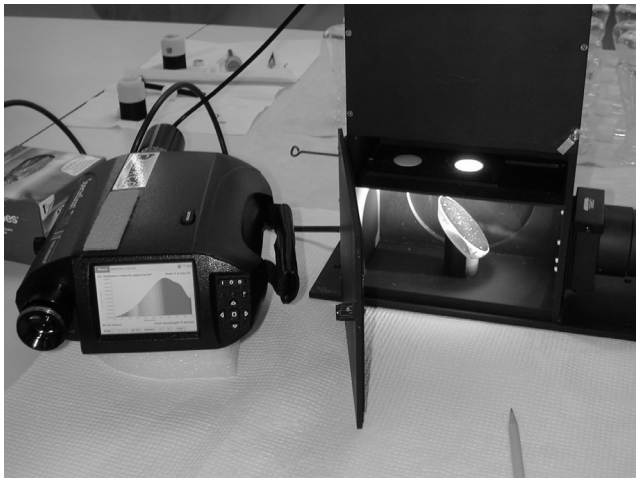

Figura 8.1.
Espectroradiometro (Spectrascan) acoplado con una fibra óptica a una caja oscura para medir la densidad óptica de los filtros por transmisión de luz.

Spectroradiometer (Spectrascan) coupled with an optical fiber to a dark box to measure the optical density of the filters by light transmission.

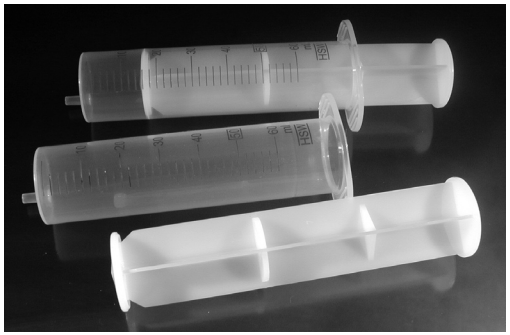

Figura 9.1.
Inyectadora de plástico utilizada para tomar las muestras de MODC. Sin émbolo de goma negra para evitar compuestos orgánicos disueltos con color.

Plastic syringe used to take CDOM samples, without the black rubber plunger to prevent dissolved organic colored compounds.

Figura 8.2.
Colocación del filtro en un embudo de filtración para ser lavado con metanol en caliente. El líquido con los pigmentos se recibe en el cilindro graduado dentro de la cámara cilíndrica de acrílico donde se hace un vacío moderado, para un paso lento del metanol por el filtro.

Filter placement in filtration funnel for hot methanol washing. The filtered liquid with pigments is captured in a graduated cylinder inside an acrylic cylindrical chamber where moderate vacuum is applied for slow filtering of the methanol.

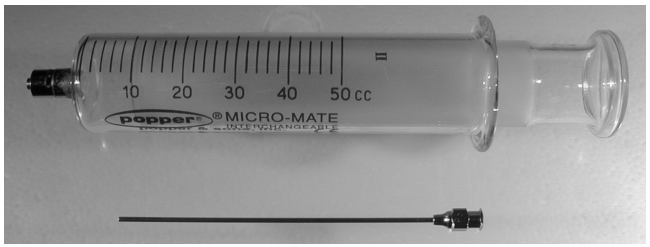

Figura 9.2.
Inyectadora de vidrio y cánula larga de acero inoxidable, para el manejo del agua de la muestra en el análisis de MODC.

Glass syringe and stainless steel tube for handling the water sample in the CDOM analysis.

Figura 10.1

Forma de manejo de los filtros calcinados al ser colocado en el soporte de filtros para la toma de muestras de COD.

Method to handle calcinated filters to be placed in the filter holder during DOC sampling.

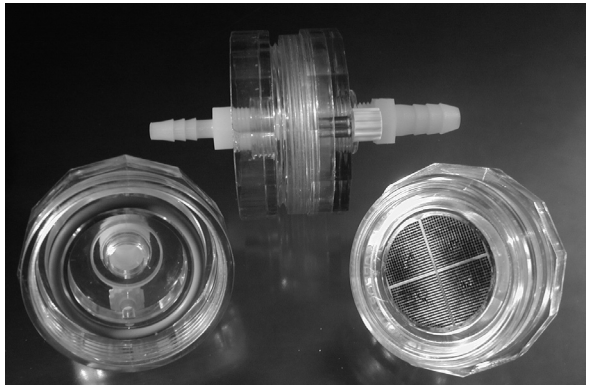

Figura 10.2

Unidad de filtración en línea armada y desarmada para filtrar la muestra de agua de mar para el análisis de COD.

Inline filtration holder, assembled and disassembled, for DOC sampling.

Figura 10.3

Forma de tomar directamente la muestra de COT de la botella Niskin.

Method to take TOC samples from the Niskin bottle

Figura 10.4

Modo de tomar la muestra con el filtro en línea para las muestras de COD.

Method to take DOC samples from the Niskin bottle.

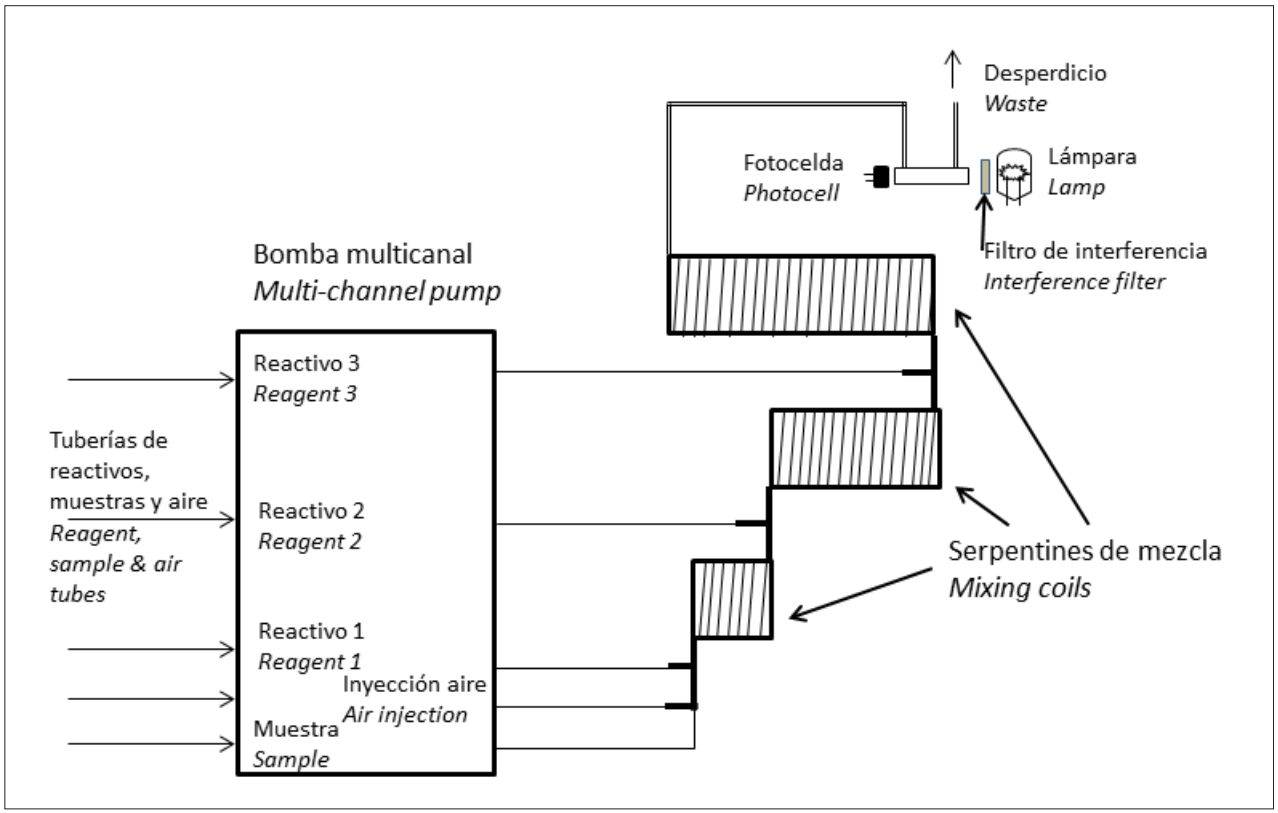

Figura 12.1.
Principales componentes de un sistema de AFC. elaborado a partir de Gordon *et al.* (2000) con permiso.
Main components of a CFA system, reproduced from Gordon et al., (2000) by permission.

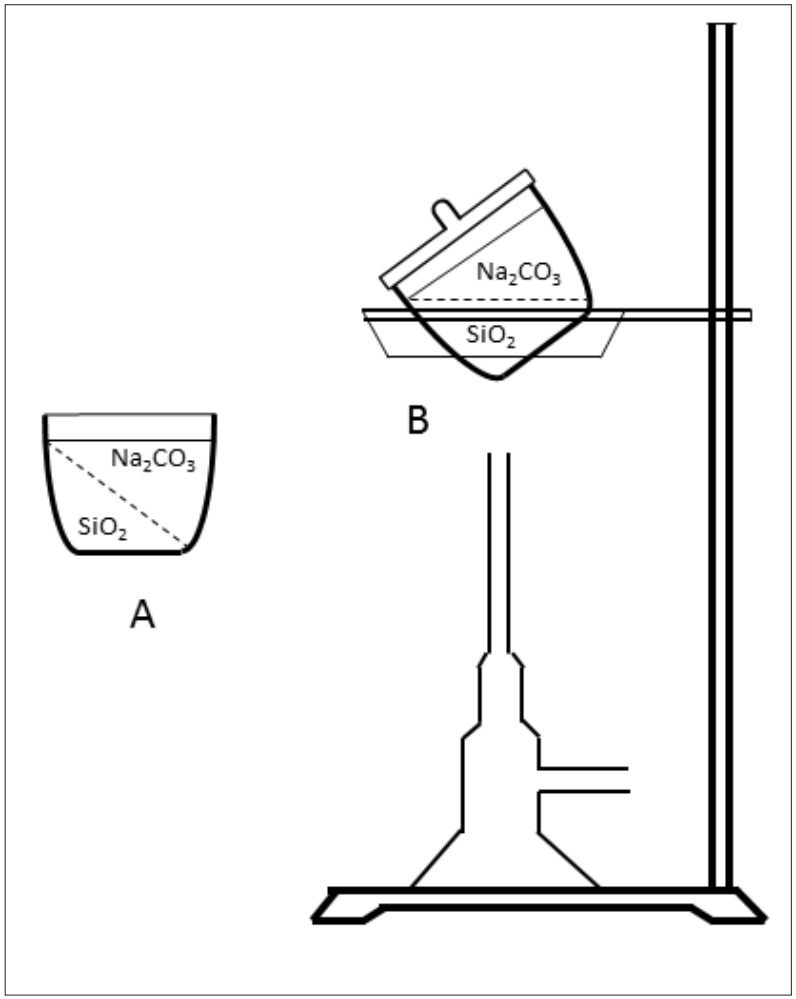

Figure 12.2.
Esquema para la preparación del estándar de ácido silícico.
Outline for the preparation of silicic acid standard.

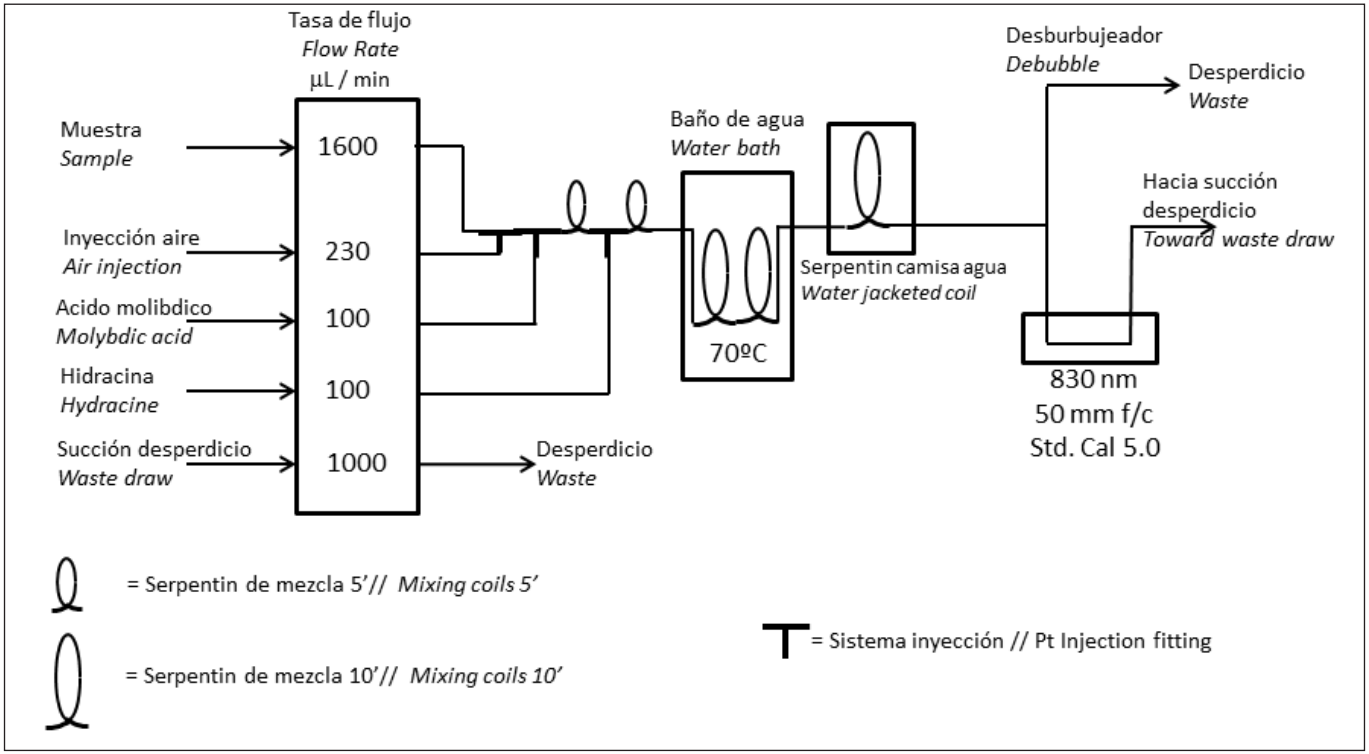

Figura 12.3.
Diagrama de flujo para el método de fosfato. Basado de Gordon *et al.* (2000) con permiso.
Flowchart for phosphate method reproduced from Gordon et al. (2000) by permission.

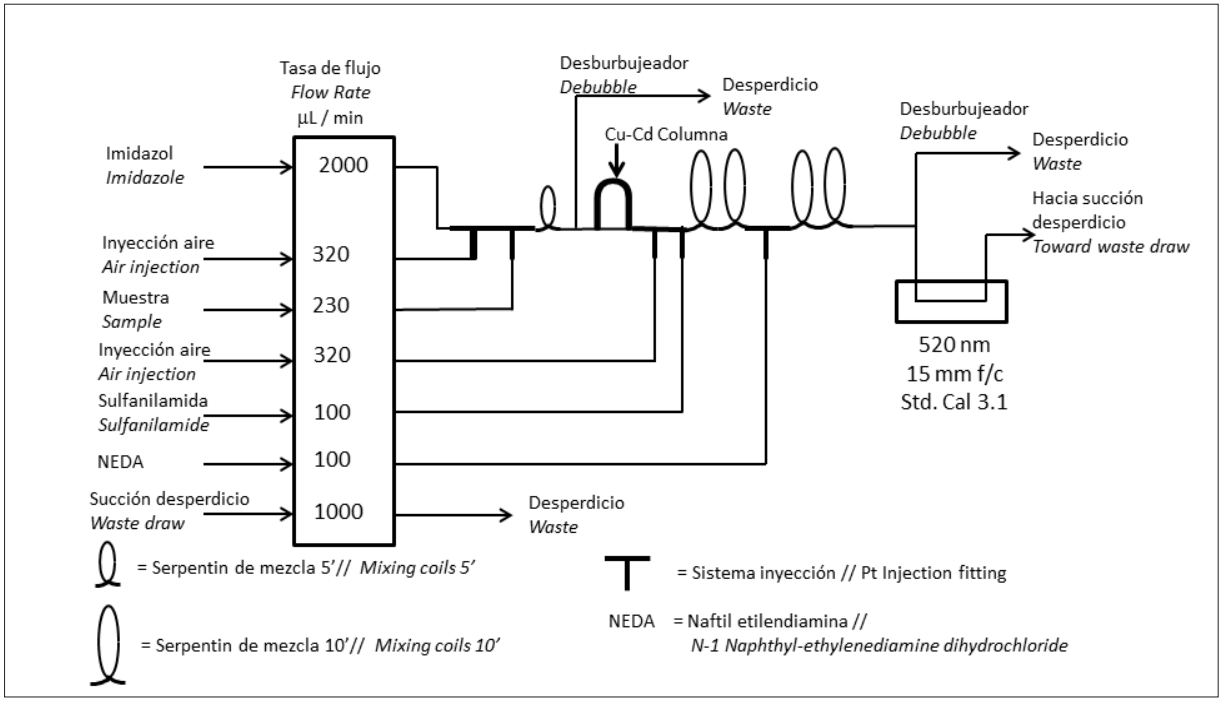

Figura 12.4.
Diagrama de flujo para el método de nitrato. Basado en Gordon *et al.* (2000) con permiso.
Flowchart for nitrate method reproduced from Gordon et al. (2000) by permission.

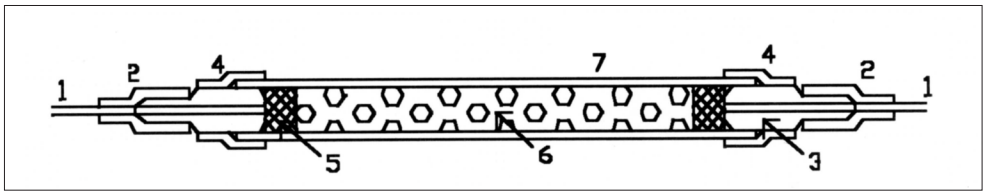

Figura 12.5
Columna de Cd-Cu empacada para uso en un AA-II. Basado en Gordon *et al.* (2000) ver texto para el significado de los números
Cd-Cu column packaged to use in an AA-II. Reproduced from Gordon et al. (2000) by permission, see text for meaning of the numbers.

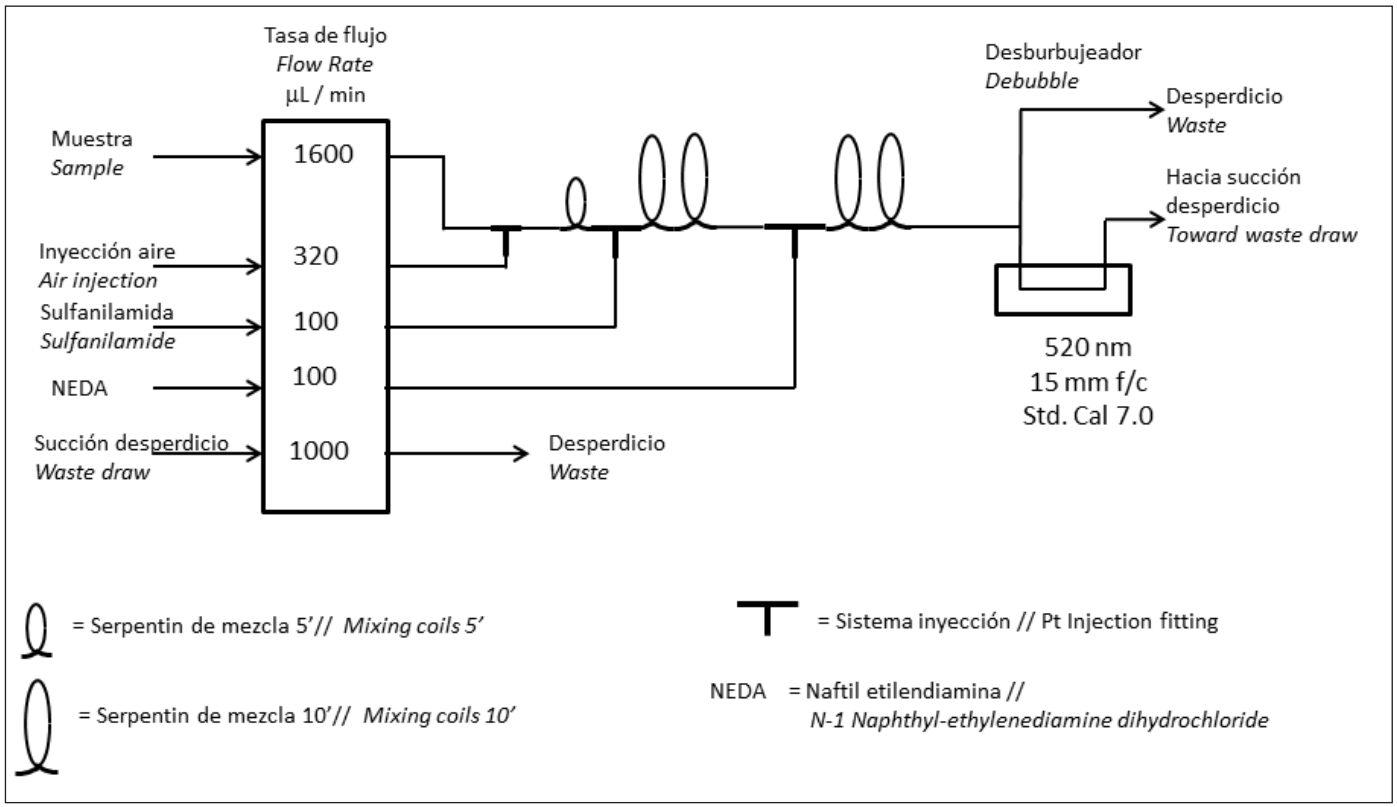

Figura 12.6
Diagrama de flujo para el método de nitrito. Basado en Gordon *et al.* (2000) con permiso.
Flowchart for nitrite method reproduced from Gordon et al. (2000) by permission.

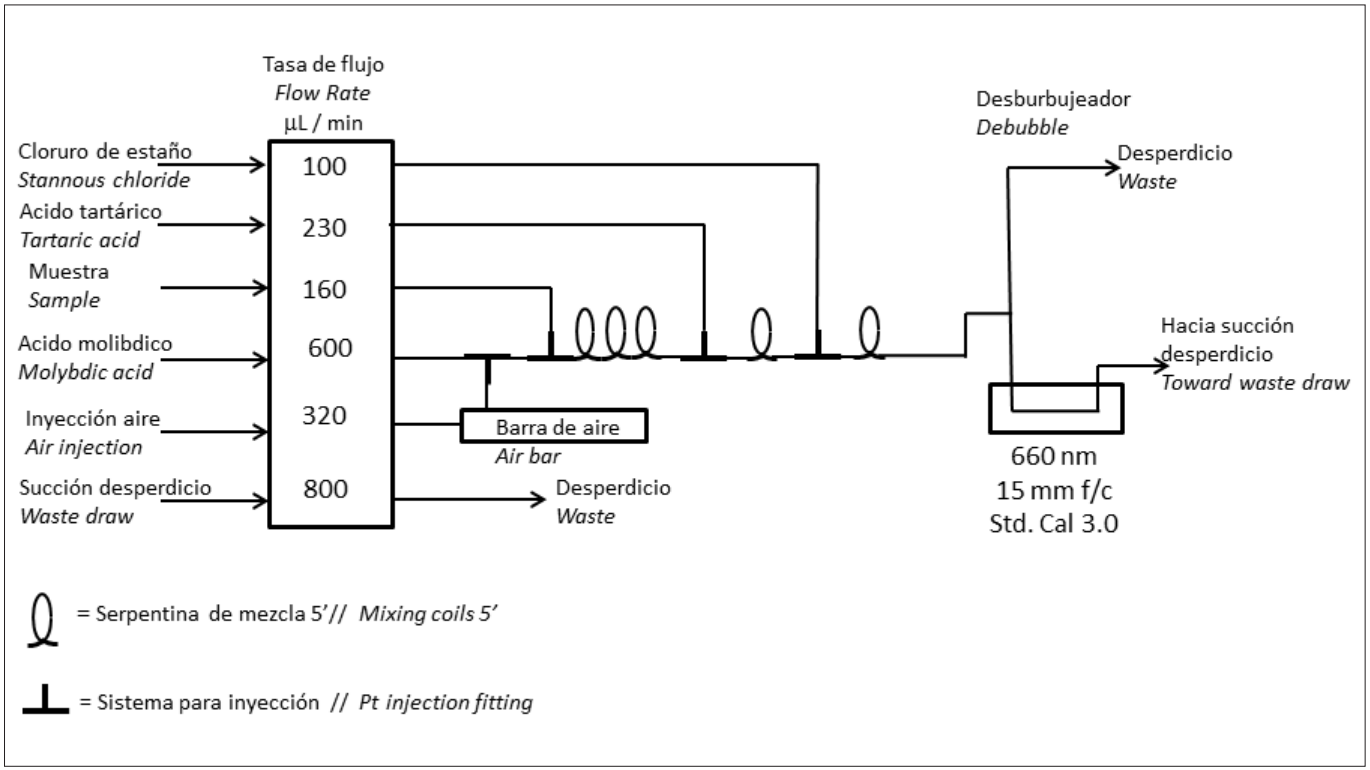

Figura 12.7.
Diagrama de flujo para el método de ácido silícico. Reproducido de Gordon *et al.* (2000) con permiso.
Flowchart for silicic acid method reproduced from Gordon et al. (2000) by permission.

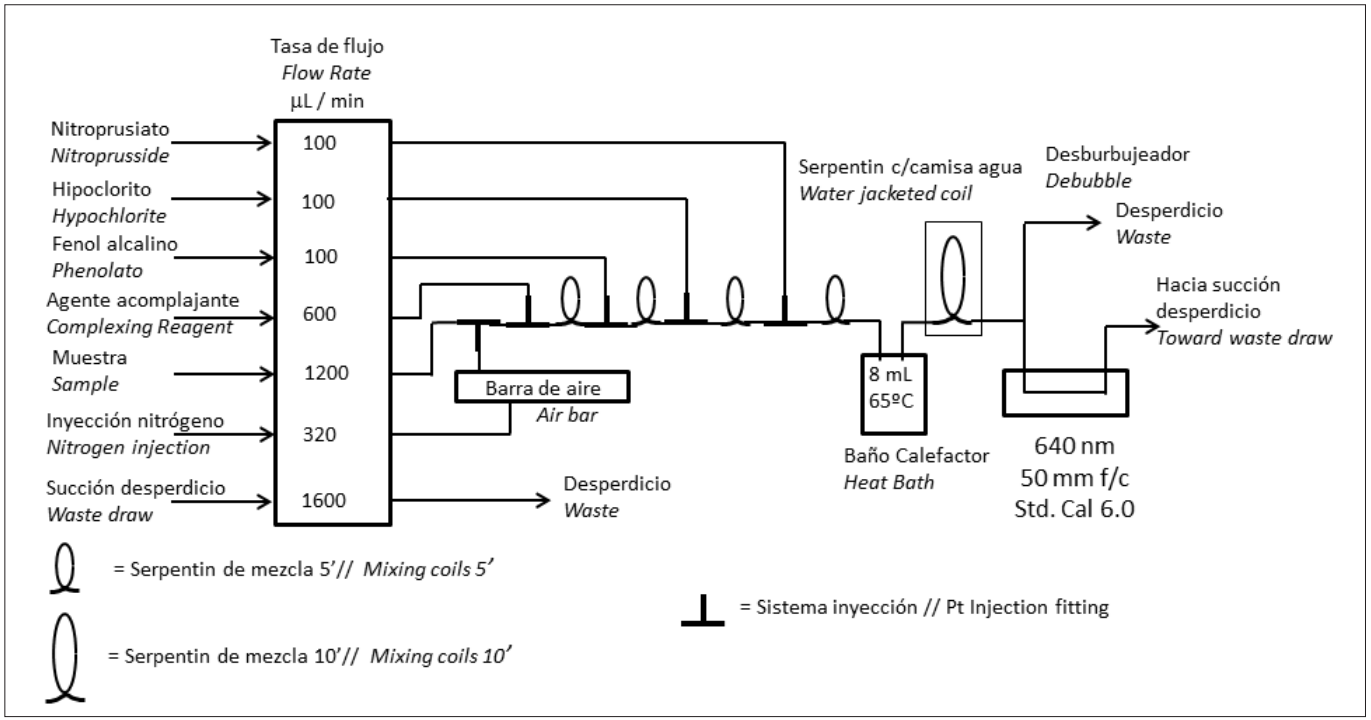

Figura 12.8.
Diagrama de flujo para el método de amonio. Basado en Gordon *et al.* (2000) con permiso.
Flowchart for ammonia method reproduced from Gordon et al. (2000) by permission.

Figura 14.1
Boya que sostiene la línea de incubación de producción primaria, y grupo de matraces de policarbonato de una de las profundidades establecidas, con tres frascos transparentes que reciben luz y uno oscurecido con un papel de aluminio.

Buoy holding the incubation line for primary production, and a group of polycarbonate flasks of one of the established depths, three transparent flasks that receive light and one wrapped in foil.

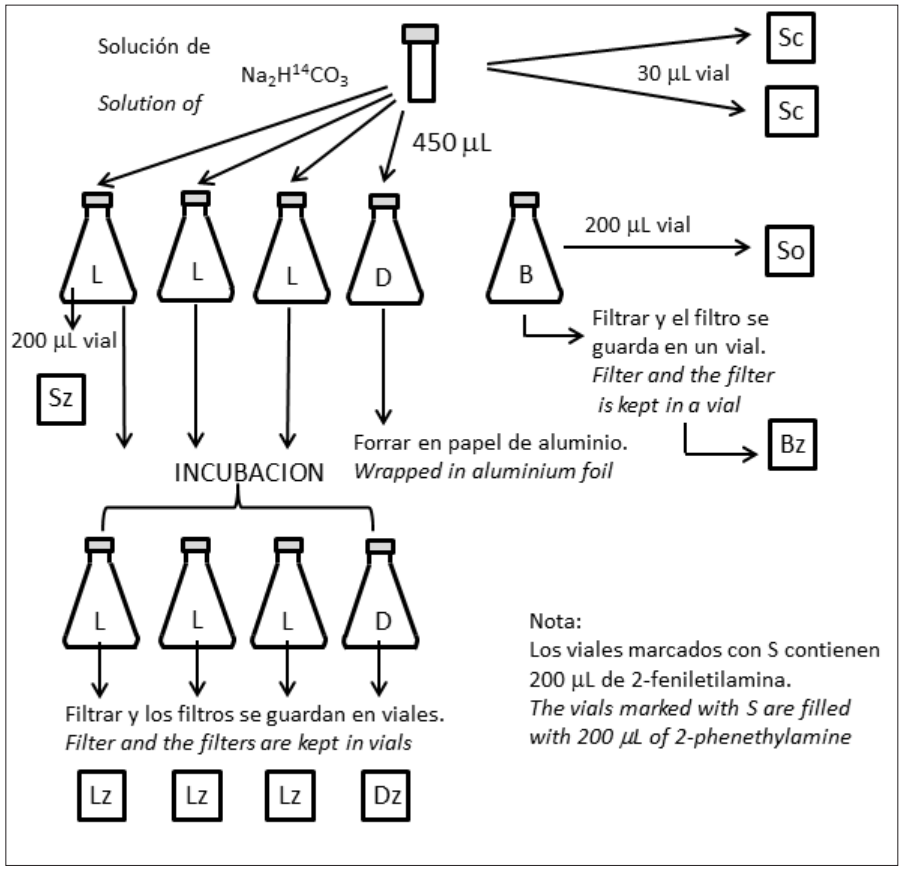

Figura 14.2.
Diagrama del procedimiento a seguir durante el análisis de producción primaria.
Diagram of the procedure to follow during primary production analysis.

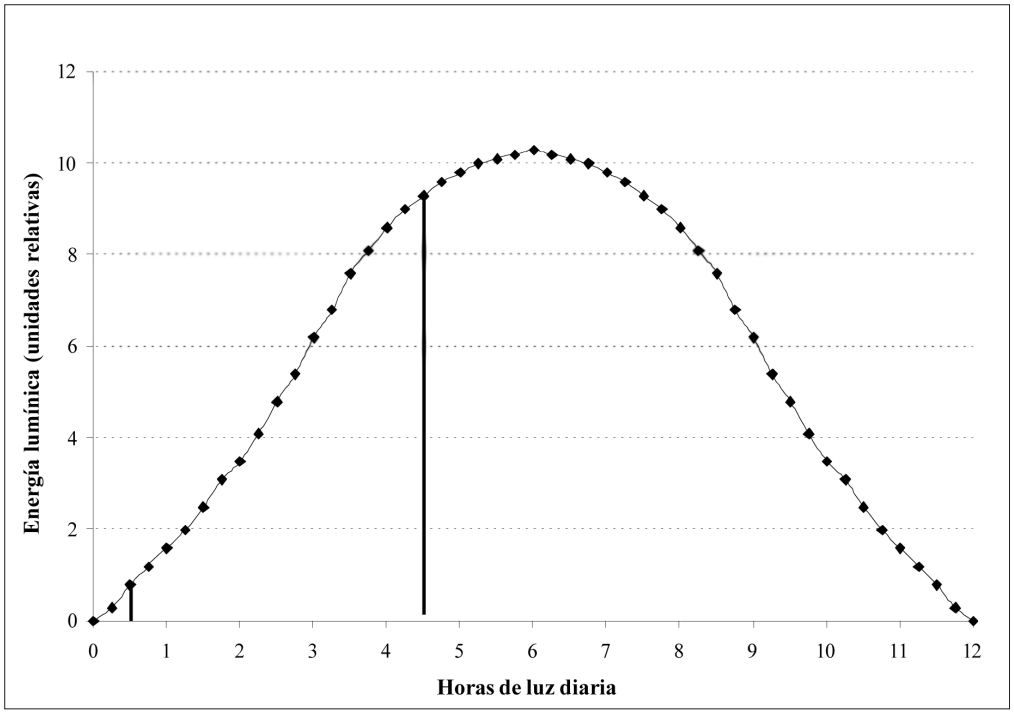

Figura 14.3.
Curva de la energía solar diaria en la estación CARIACO. Las líneas verticales limitan el período establecido en CARIACO.

Curve of daily solar radiation at the CARIACO station. The vertical line indicates the period of incubation used in CARIACO.

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