Standard Operating Procedure
Long Island Sound Survey (LISS)
Nutrients Laboratory

Prepared by:
Name: Steph Kexel **Signature**: ______________________________ **Title**: __________________________ **Date**: ________________

Approved by:
Name: Chris Perkins **Signature**: ______________________________ **Title**: __________________________ **Date**: ________________

CESE
Center for Environmental Sciences and Engineering
Building 4 Annex
3107 Horsebarn Hill Road; U-4210
University of Connecticut
Storrs, CT 06269-5210

State of CT: PH-0778 – EPA: CT01022

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Introduction

This laboratory operation manual is a document delineating the procedures used by the Center for Environmental Sciences and Engineering's (CESE) Nutrient Laboratory at the University of Connecticut to analyze seawater samples for the Connecticut Department of Energy and Environmental Protection's (CT DEEP) Bureau of Water Management Long Island Sound Study (LISS).

Explanation of Tables

Table 1-1 presents a list of major equipment used for the analysis of nutrients. Additional equipment and materials used on this project are listed in the individual standard operating procedures (SOPs).

Table 1-2 summarizes the analytical methods used in the analysis of seawater samples.

Table 1-3 summarizes the quality control (QC) checks required for each group of analyses.

Table 1-4 summarizes the precision and accuracy objectives of the laboratory methods. The calibration verifications rely on analysis of samples traceable to the National Institute of Standards and Technology (NIST) or the Environmental Protection Agency (EPA). These are used as controlling elements for the methods, and ensure that the calibration curve used is representative for the entire analytical run, and that the precision meets the requirements shown in Table 1-4. All QC checks are stored in hard-copy form, with the results of each sample delivery group (SDG). Data reports can be obtained with a sample delivery group number to allow quality assurance (QA) review of any sample analysis and evaluation of any of the associated Quality Assurance checks.

Table 1-5 shows the organization of the monthly QC/QA and quarterly reports submitted to the CT DEEP.

Table 1-1—Laboratory Equipment in Use

Equipment	Manufacturer	Model	Serial #
QuickChem	Lachat	8500	040600000007
Total Organic Carbon Analyzer	Shimadzu	TOC-L CPH	H54214900076AE
Fluorometer	Turner	Trilogy	720000403
CHN Analyzer	Perkin Elmer	Series 2	24N1021602
Microgram Balance	Mettler	XP26	1128431375
Drying Oven	Fisher	Isotemp 176	131
Autoclave (2)	Market Forge	STM-E	
Muffle Furnace	Fisher Scientific	Isotemp 550-126	1511071280908

Table 1-2—Analytical Methods of Analysis

Parameter	MDL	Instrument	Method
Ammonia (NH_3)	0.004 mg/L	Lachat Quick Chem	EPA 350.1
Nitrate + Nitrite (NO_x)	0.002 mg/L	Lachat Quick Chem	EPA 353.2
Chlorophyll-a (CHL-a)	0.100 µg/L Calculated	Turner Fluorometer	EPA 445.0
Ortho-phosphate (DIP)	0.001 mg/L	Lachat Quick Chem	EPA 365.1
Dissolved Silica (SiO_2)	0.005 mg/L	Lachat Quick Chem	EPA 370.1
Dissolved Organic Carbon (DOC)	0.300 mg/L	Shimadzu TOC-L High Temperature Combustion	EPA 415.1
Total Dissolved Phosphorus (TDP)	0.001 mg/L	Lachat Quick Chem	EPA 365.1
Total Dissolved Nitrogen (TDN)	0.014 mg/L	Lachat Quick Chem	EPA 353.2
Particulate Nitrogen (PN)	0.003 mg/L	CHN High Temperature Combustion	EPA 440
Particulate Phosphorus (PP)	0.0002 mg/L Calculated	Lachat Quick Chem	EPA 365.1 HCl extraction
Particulate Carbon (PC)	0.021 mg/L	CHN High Temperature Combustion	EPA 440
Biogenic Silica (Bio SiO_2)	0.001 mg/L	Lachat Quick Chem	EPA 370.1 NaOH Extraction
Total Suspended Solids (TSS)	2 mg/L	Gravimetric	EPA 160.2

Table 1-3—Summary of Quality Control Checks

NH_3, NO_X, DIP, SiO_2, TDN, TDP, Bio SiO_2 and PP

1. Calibration Curve, five to seven points.
2. One method blank every 10 samples (where applicable).
3. Calibration Curve Verification every 10 samples.
4. Calibration Blank Verification every 10 samples.
5. Spike Recovery analysis every 10 samples.
6. Laboratory Duplicate analysis every 10 samples.

DOC

1. Calibration Curve, 4 points.
2. Calibration Curve Verification every 10 samples.
3. Calibration Blank Verification every 10 samples.
4. Spike Recovery analysis every 10 samples.
5. Laboratory Duplicate analysis every 10 samples.

PN/PC

1. Calibration Curve, one point.
2. One method blank per 10 samples.
3. One K-Factor every 10 samples.

TSS

1. Analysis of NIST weights to verify calibration.

CHL-a

1. Calibration Curve, three points
2. One method blank per 20 samples
3. Calibration Verification every 20 samples
4. Spike Recovery analysis every delivery group.

Table 1-4—Laboratory Precision and Accuracy Objectives

Parameter	Method	Relative % Difference	% Recovery
NH_3	EPA 350.1	<15	85-115
NO_x	EPA 353.2	<15	85-115
DIP	EPA 365.1	<15	85-115
SiO_2	EPA 370.1	<15	85-115
TDP	EPA 365.1	<15	85-115
TDN	EPA 353.2	<15	85-115
DOC	EPA 415.1	<15	85-115
PN	EPA 440.0	NA	85-115
PC	EPA 440.0	NA	85-115
Bio SiO_2	EPA 370.1	<15	85-115
PP	EPA 365.1	<15	85-115
TSS	EPA 160.2	NA	85-115
CHL-a	EPA 445.0	NA	85-115

Table 1-5—Organization of Monthly Data/QC Report

The monthly data report is submitted, via email, in an electronic format to CTDEEP, within 60 days of the receipt of the final sample collected during each month. All narratives, data and quality control results are submitted as PDF files, while the data are also submitted in an excel spreadsheet in a format provided by CTEDEEP. A copy of the final signed chain of custody will be scanned and emailed to CTDEEP. The monthly data reporting consists of:

1. Project Narrative
   This document contains a discussion of any problems with analytical results, quality control, sample handling, labeling or holding times, and if applicable, any corrective action taken.

2. Data Reports
   These consist of 2 (i.e. nutrients and biological oxygen demand) PDF reports for data collected for each month.

3. Quality Assurance Report
   This document consists of a table for each parameter, and details holding times, analysis date and QC results (where applicable) for:
   a. Initial and Continuing Blank Verification
   b. Initial and Continuing Calibration Verification
   c. Laboratory Duplicate Analyses
   d. Field Blank Analyses
   e. Field Duplicate Analyses
   f. Laboratory Blank Analyses
   g. Laboratory Spike Analyses

Procedure Prior to Sample Analysis

Total Suspended Solids

Pre-weighed filters are available in the laboratory and are used by CT DEEP sampling personnel for LISS TSS samples. The filters are supplied by Environmental Express. (ProWeigh Filters cat # F93447MM). Each 47mm filter comes with an individual tin on which is printed the filter's identification number and weight and is certified for accuracy by the manufacturer.

Particulate Carbon and Nitrogen Filter Pad Preparation

Loosely arrange 25 mm, 0.7µm glass fiber filter pads in crucibles and place the crucibles in the muffle furnace for combustion at 500°C for 1 hour. The crucibles are then cooled, transferred to a desiccator and allowed to equilibrate at room temperature. The filter pads are then transferred to clean, dust free plastic bottles, and sealed for delivery to the CT DEEP.

Prior to packing, acceptance testing must be performed on each batch of filters using 2 filters per batch of 100 filters. The filters are analyzed for particulate carbon and nitrogen, and if the analysis shows carbon or nitrogen contamination the lot must be discarded and a new lot prepared. The analysis procedure must then be repeated.

Biogenic Silica Filter Pad Preparation

No preparation is required for the 47 mm, 0.4µm poly-carbonate membrane filter pad.

Particulate Phosphorus Filter Pad Preparation

No preparation is required for the 47 mm, 0.7µm glass microfiber filter pads.

Chlorophyll-a Filter Pad Preparation

No preparation is required for the 0.7µm, 25mm glass fiber filter pad.

Preparation of 250mL and 125mL Polyethylene Sample Bottles.

Acid soak with dilute hydrochloric acid (HCl) for at least 15 minutes by fully submerging and ensuring the bottle is void of air bubbles in an acid bath.

Rinse 3 times with deionized water. Fill with completely with DI and swirl to ensure all inside surfaces are rinsed with DI. Shake out DI in between rinses to reduce to a minimum the carryover of DI from rinse to rinse.

Place in clean drying area with open end towards the countertop.

Biogenic Silica Centrifuge Tubes

No preparation or washing is required.

Procedure for Sample Receipt

After delivery and signing of chain of custody sheets, information is taken from the field data sheets and entered the Laboratory Information Management System (LIMS). Each sample is assigned a unique laboratory identification number which consists of a group identification number, which is a continuously increasing number generated by the LIMS, and a sample ID that increases in ascending order based on the arrival time at the laboratory. The laboratory group number is designated as the sample delivery group (SDG) number, which is then used for reporting purposes.

Information recorded into the LIMS includes date of collection, date of delivery, parameters requested and client contact information.

After the samples are grouped, labels are placed on the containers, and the samples are placed in appropriate storage until analysis can be performed.

Sample Requirement and Preservation

Test	Supply Requirement	Replicate	Storage
PC/PN	25mm GF/F	Duplicate	Aluminum Foil/Frozen
PP	47mm GF/F	Duplicate	Aluminum Foil/Frozen
TSS	47mm GF/F	Single	Proweigh Pre-labeled Tin
CHl-a	25mm GF/F	Duplicate	Aluminum Foil/Frozen
$BioSiO_2$	47mm PCM/F	Single	Centrifuge Tube/Frozen
NO_X-N, PO_4^{3-}-P NH_3-N, TDP TDN, DOC	250mL Bottle	Single	Frozen
SiO_2	125mL Bottle	Single	Refrigerate 4ºC (do not freeze)

The Lachat Quick Chem 8500

Scope and Application

The Lachat QuickChem 8500 is a wet-chemistry continuous flow-analyzer that is used in laboratories for the automation of complex chemical reactions. It uses the principle of continuous flow-analysis (CFA) for fully automatic sample analysis. Samples are mixed with reagents in a continuously flowing stream. In the Nutrients Laboratory, chemistries currently run on the Lachat for the LISS are as follows:

Nitrate + Nitrite as N
Total Dissolved Nitrogen as N
Ammonia as N
Dissolved Inorganic Phosphorus as P
Total Dissolved Phosphorus as P
Particulate Phosphorus as P
Silica as SiO_2
Biogenic SiO_2

The Lachat consists of 4 main components. These are the XYZ Sampler, the reagent pump, the Chemistry manifold with detectors, and the computer with monitor and the printer.

The XYZ Sampler is a computer controlled random access sampler designed to automatically introduce samples in a preprogrammed sequence into the analytical system.

The reagent pump is in contact with peristaltic pump tubing, spinning at a predetermined speed to provide precise volume delivery of sample and reagents. Pump tubes and lines are made of flexible PVC. The individual pump platens hold the pump tubes in place and ensure correct pressure against the pump rollers for positive sample/reagent flow. At the rear of the pump are the power button, the standby button and the buttons to vary pump speed.

Chemical reactions are carried out on the chemistry module. The samples are injected into a continuous “carrier” stream. The carrier is free of the analyte being measured and thus serves as a baseline. The chemistry manifolds contain the required components to complete color development. Samples are inserted into the carrier stream; develop color when mixed with reagents, and stand out in absorbency value against the carrier baseline background. The information regarding absorbency value collected at the detector head is fed to the computer of the system unit and is displayed on the monitor screen. Samples show as peaks against the flat baseline. The software uses the peak area to determine the concentration of analyte.

There are four channels on the Chemistry Module. The channel that is closest to the analyst when looking at the instrument is channel 4. This channel runs Nitrite. Channel 3 runs Total Nitrogen and Nitrate + Nitrite. Channel 2 runs Dissolved Inorganic Phosphorus, Total Dissolved Phosphorus, and Particulate Phosphorus. This channel is switched out for the SiO_2 analysis. Channel one runs Ammonia.

Omnion version 3.0 is the software that controls the Lachat. Results are calculated from peak area.

References

QuickChem 8500 FIA Automated Ion Analyzer Operation Manual. Lachat Instruments, 2004.

Software User Guide: Omnion 3.0. Hach Company, 2004.

Associated SOP's

Refer to "SOP" titled "Hazardous Waste SOP" for proper waste disposal.

Refer to the notebook titled "SOP's" located in the nutrients laboratory.

Safety

Waste effluents must be kept separately from each other. The waste streams from the phosphorous manifold and the nitrate/nitrite manifold must not be combined.

Be sure to check each specific method for hazards related to wastes and reagent composition.

Refer to the University of Connecticut's Environmental Health and Safety chemical health and safety web page at:

http://www.ehs.uconn.edu/ppp/index.php

A hard copy of the Chemical Hygiene Plan can be found on the notebook rack located on the lab bench. Also refer to the Hazardous Waste SOP.

Materials

Chemistry Module
Peristaltic Pump
XYZ Auto-sampler
Computer
Printer
See individual methods for each analyte for other materials

Procedure

Instrument Start-up Procedure

Turn on the auto sampler, place the pump platens in place and start the pump. Turn on the power to the chemistry module and boot up the computer.

Activate the software by selecting "Omnion" icon on the desktop. Click "Open" at the top of the menu.

Select the correct method: Nitrate + Nitrite, TDN, TDP etc. A template run will pop up with all of the pertinent QC, Standard, Blank and spike information. If nothing needs to be changed, enter the sample ID's for each corresponding cup number. Acceptable limits are already programmed into the software.

To change quality assurance limits, activate the run properties window and click on the tab for "sample". This will bring up the data quality management (DQM) specifics and they may be changed here. Be sure that the proper sample is highlighted in the run worksheet.

Methods have already been developed that utilize the Refractive Index Correction in the software for saltwater matrices. It is not necessary to matrix match standards and carrier to samples as the software will select an area of the peak to integrate that omits the refractive index dip that occurs at the start of a peak.

Make sure that the proper wavelength of filter is in the proper detector location (see individual methods for wavelengths).

Make sure that the proper length sample loop is attached between valve positions 1 and 4 (see individual methods).

Ensure that effluent lines are in the proper hazardous waste receptacle.

After 10 minutes of rinsing with deionized Water, put the reagents in their proper reagent bottles. Let reagents run through lines for at least 10 minutes before proceeding.

If running the NO_x manifold, make sure the cadmium column is active at this point.

If running ammonia, put reagent lines in this order: buffer, phenol, nitroprusside and bleach, letting each flow through the manifold before adding another line.

Run Set-up Procedure

Select the run worksheet window to make it active and then enter the sample calibration and sample IDs. When a sample is highlighted select the “Sample” tab at the top of the run properties window to change acceptable limits.

The run must have this order:

Type	Concentration	Location
Calibrant	Calibrant	cup position 346
Calibrant	Calibrant	cup position 347
Calibrant	Calibrant	cup position 348
Calibrant	Calibrant	cup position 349
Calibrant	Calibrant	cup position 350
Calibrant	Calibrant	cup position 351

To add samples, QCs, blanks, spikes or calibrants, click on the corresponding pre-entered value to highlight lines and copy and past them to the desired location. All of the DQM test requirements will transfer with the copied cells.

New lines may be inserted into the software by clicking on the sample number and right clicking on the mouse and either “insert sample(s)” or at the end of a sheet you can add lines by right clicking on the mouse and “append one or many”.

Any run may be modified while running samples as long as it’s not close to the end of the run. Once a run is completed, you must re-calibrate and set up a new run.

At the end of the run, the last sample must be PQL.

The software generates a filename for each run created. The filename will be OM DD MM YYYY HH MM SS AM or PM.OMN. If there are multiple runs for the same day, the date will be the same, but the hour/min/second will change. Be sure to export the run information under the file tab to print the run log then put it in the run logbook.

Starting a Run

Select the green arrow “start” button on the top of the main menu tool bar.

During the Run

After the calibration has been completed, check the correlation coefficient. To view the calibration graph, click on the chart recorder window's chart icon at the left side of the window.

If the software is not capturing the entire peak in the peak expectation window, wait for 2 full peaks to appear on the screen and stop the run. When prompted to stop the run, select "yes". Then double click on the peak and the peak windows will appear. Click and drag the sides of the peak expectation window to encompass the entire peak. Right click on the peak and select "Adjust Peak Expectation Window". This should capture the entire peak. Once this step is completed, the software is ready to continue with the calibration.

Select the green arrow "start" button to begin the run again. Ensure that there is enough standard solution in the sample cups to continue and verify that the calibration coefficient is 0.995 or greater before continuing.

Once calibration passes, ensure that the QC and BLK pass and continue with the run.

See each individual method for proper spike concentrations.

After the Run is Complete

Although this is a rare occasion, it may be necessary to delete a point from the calibration curve. Only one point may be deleted from the curve, and once the point is deleted, the correlation coefficient must yield a value of 0.997 or greater. If a point is deleted, the deleted standard concentration must be re-made and re-run at the end of the run to verify that the standard was made incorrectly or that instrument problems precluded an accurate sample analysis. If the lowest point (the PQL) is deleted from the curve, the next highest standard becomes the PQL and all data between this higher point and the MDL must be flagged.

To review data, select the yellow "custom report format" button at the top of the main menu. On the table tab, ensure that the Cup No. and Manual Dilution Factor boxes are checked off.

In the calculations tab, ensure that under sample preparation, the multiply concentration by manual dilution factor box is checked off.

In the charts tab, ensure that the calibration, DQM tests, and channel data display boxes are all checked off and that the "show 10 peaks per chart" for all peaks is checked off.

Print the report, then double click on the run worksheet so that the entire run is highlighted, then click on "run" at the top of the screen, and then select "export worksheet data". This will print the run log to put in the proper run logbook.

Shutting Down the Instrument

If running NO_x, turn the cadmium column off-line.

Place all reagent lines in deionized water for 10 minutes, pulling reagent lines out of solution in the opposite order that they were placed in reagents (see individual methods for correct order of reagent line orders). Pump air through the lines until no liquid can be seen running through the manifolds.

Dump out the sample probe rinse bottle and sample cups. Shut down the computer. Shut off the chemistry module. Shut the pump off and remove the platens. Shut off the auto sampler.

Particulate Carbon and Particulate Nitrogen

Scope and Application

This SOP is used for analysis of particulate carbon (PC) and particulate nitrogen (PN) in filtered seawater samples. The holding time is 28 days.

The CHN analyzer uses a combustion method to convert the sample elements to simple gases (CO_2, H_2O, and N_2). The sample is first oxidized in a pure oxygen environment using classical reagents.

The products that are produced in the combustion zone include CO_2, H_2O, and N_2. Elements, such as halogens and sulfur, are removed by scrubbing reagents in the combustion zone. The resulting gases are homogenized and controlled to exact conditions of pressure, temperature, and volume.

The homogenized gases are allowed to de-pressurize through a column where they are separated in a stepwise, steady-state manner and detected as a function of their thermal conductivities (Perkin Elmer CHNS/O Manual).

The Method Detection Limit (MDL) and Practical Quantitation Limit (PQL) for this analysis are determined yearly. For current MDL and PQL limits, see the chart entitled Method Detection Limit Determination.

References

CHNS/O Elemental Analyzer Operation Manual. Perkin Elmer, Revision D. Shelton, CT 06484.

Associated SOP's

Refer to "SOP" titled "Hazardous Waste SOP" for proper waste disposal.

Refer to the notebook titled "SOP's" located in the nutrients laboratory.

Safety

Combustion and reduction tubes are treated as hazardous waste, are labeled with contents and placed in hazardous waste bin. When packing tubes, use proper protective gear and work in hood as reagents are hazardous.

Refer to the University of Connecticut's Environmental Health and Safety chemical health and safety web page at:

http://www.ehs.uconn.edu/ppp/index.php

A hard copy of the Chemical Hygiene Plan can be found on the notebook rack located on the lab bench. Also refer to the Hazardous Waste SOP.

Materials

Perkin Elmer CHNS/O Elemental Analyzer 2400 Series II
Combustion and Reduction Tubes
Reagents (see Perkin Elmer Manual)

Procedure

Starting up the Instrument

Begin by turning on the three tanks of gases (helium, nitrogen and compressed air). Turn on the computer. Power up the instrument by flipping the switch on the bottom-right hand side of the instrument.

The instrument will prompt to enter the time, date (expressed as d/m/yr) and then will go through a series of pressure checks. Throughout this SOP whenever a button is depressed on the instrument, the button identification will be within <brackets>.

The instrument will ask for an operator id. Enter initials and press <enter>. To write letters with the number keypad, press <.> then the corresponding number associated with the letter. The code is affixed to the front of the CHN.

The instrument will now display the fill pressure. Depress <enter>.

Next, the run counter will display run counts for the combustion tube, the reduction tube and the vial receptacle. Press <enter> to scroll through each of these. These options are to track the number of runs passed through each section, and when the limit is reached, an alarm will sound. The vial receptacle will need to be removed and emptied after 200-300 runs. The reduction tube will need replacing after approximately 175 runs. Lastly, the combustion tube should be replaced after approximately 1000 runs. These values may be entered into the run counter to keep track of when tubes need changing. Entering these values is only a guide and is not necessary. The analyst must assess the data to ensure that tubes are consistently producing accurate results.

Next, the instrument will display the furnace temperatures for each zone. Depress <enter> to scroll through the temperatures. They will always remain set at 925°C for the combustion temperature and 640°C for the reduction temperature.

Next the instrument will run through a self check and will pressurize to about 700mmHg.

The instrument will prompt if a helium and oxygen purge should be completed. Depress <N> for both. The instrument will be in standby mode. Purge will happen later.

Next direct the instrument to dump data to the computer by pressing <parameter 5> for the data folder, then <parameter 26> <yes>. Then <parameter 40—yes—yes> and <parameter 41—yes>.

If the combustion and reduction tubes have been changed, a leak check must be completed on the system. Press <diagnostics> and <2> for gas, then <1> for leak checks. Press <1> <enter> for the code of the mixing chamber. If the tubes are inserted properly, the leak test will pass. The pressure will increase to about 660mmHg and should hold steady for the duration of the leak check. After passing, depress <diagnostics> and the instrument will read standby.

Press <diagnostics> again to test the combustion tube pressure. The pressure should read about 770mmHg and should be stable for the duration of the leak check. Press <2> for gases and <1> for a leak check. Press <2>, which is the code for the combustion tube leak check. Again, be sure that the test passes before continuing. If the test does not pass, re-insert the tube and check all

fittings. If this still does not fix the problem, check all o-rings and replace if necessary and re-insert the tubes. If this still doesn't work, contact technical support.

Depress <diagnostics> until the instrument is in standby mode. Turn the furnace on if not already on. To check the furnace temperature, press <monitor> and <N> so the printer does not print. Next, press <1> <enter> to check the furnace temperature of the combustion zone. If the temperature is rising, then the furnace is on. Press <monitor> to return to standby mode. To turn the furnace on, press <parameters> and option code <12> <enter> and observe which flashing. Turn on by pressing <1>. Press <12> <enter> again to ensure that the number 1 is flashing, signaling that the furnace is in the on position. This is important as sometimes the furnace does not turn on during the first attempt.

Press the <parameters> key until the instrument is in the standby mode.

Check that the furnace temperature is rising by repeating the monitor step above.

When the furnace reaches full temperature, purge the gases by pressing <purge gas> <Y> for helium and <300> <enter> for a 300 second purge. Then enter <Y> for the oxygen purge and <30> <enter> for a 30 second purge time.

Now the instrument must warm up for at least 4 hours, but it is preferable to warm it up overnight.

Beginning a run

Once heated, blanks must be run until they reproduce themselves. To run a blank, press <single run> then <1> for a blank sample and the instrument will prompt for the number of runs. When starting with fresh tubes, 10-15 blanks are appropriate, so enter <10> then <enter>. The instrument will be in standby mode. Press <start> and the instrument will begin running the blanks. Blank reproduction must be within:

- Carbon +/- 30
- Hydrogen +/- 100
- Nitrogen +/- 16

Conditioning New Tubes

When new tubes are inserted in the instrument, they must be conditioned with k-factors. Run a blank, <single run> <1> <start>. When completed, run a k-factor as a sample by pressing <single run> <3> for sample, <00> <enter> for sample ID, then <start>. Next run another blank and another k-factor as sample, then one last blank (see above).

Running Blanks

The instrument must be blanked out before analysis. Run 10-15 blanks if the instrument has been started after a period of shutdown. Initially, the blanks will be air blanks, so that the high concentrations of analytes will be purged from the system. Then run 5 tin disk blanks. From now on, any time a blank is run, it MUST be a tin disk.

The system does not average blank runs consecutively. Instead, it involves comparisons among three blank values from the latest run, the prior run, and the current running average resulting in three possible scenarios: (1) The value of the latest run is compared with the current running average. (2) When the difference between these two values meets the specified criterion limit, the values are averaged and become the NEW running average. Otherwise, a second comparison is made. (3) The value of the latest run is compared with the value of the prior run. If the difference between these two values meets the same specified criterion limit, these two values are averaged

and become the NEW running average. If neither of these above meets the specified criterion limit, the current running average remains in effect.

Calibrating the Instrument

The instrument is calibrated using a 1-point calibration using an acetanilide k-factor. K-factors are weighed on the microbalance and weights are recorded in the k-factor logbook. The ideal weight for k-factors is around 2.0mg +/- 0.5mg.

The run sequence for calibrating the instrument is as follows:

- Blank tin disk<single run> <1> <1> <start>
- K-factor <single run> <2> <1> (for standard S1), enter weight of k-factor <2.000> <start>
- Blank tin disk <single run> <1> <1> <start>
- K-factor <single run> <2> <1> (for standard S1), enter weight of k-factor <2.000> <start>
- K-factor <single run> <2> <1> (for standard S1), enter weight of k-factor <2.000> <start>
- K-factor <single run> <2> <1> (for standard S1), enter weight of k-factor <2.000> <start>

Run K-factors until these limits are met. It usually only requires three consecutive k-factor runs and then proceed to the next blank.

- Blank tin disk <single run> <1> <1> <start>
- K-factor <single run> <2> <1> (for standard S1), enter weight of k-factor <2.000> <start>

The k-factor values are generally within the following expected range:

- C 16.5 +/-3.5
- H 50.0 +/-20.0
- N 6.0 +/-3.0

After calibrating, set the instrument up to run water filters by pressing <parameters> <32>. The instrument will ask if running water samples. Press <Y>. The instrument will ask if running ppm or ppb. The 2 should be flashing indicating that ppb has been selected. When running PC/PN water filters, it is not possible to put the instrument into ppm mode, even though these are the units reported to the client. The instrument will always round up anything below 1.0ppm. Press <enter>. Next, the amount of water filtered will be displayed. We keep the instrument set at 200mL so press <enter>. If water concentrations differ, change to appropriate volume filtered. Press <parameters> until the instrument reads standby.

Running Samples in an Auto Run

Samples may be run individually by the method described above or by setting up an auto run. The sample tray holds 60 samples but as the tray rotates, additional samples may be added to the auto run. Be very careful entering auto run data because once entered, changing the information is not possible. If an error is made, the entire auto run must be deleted and re-entered.

To set up an auto run, place samples in appropriate tray position. To clear previous auto run information, select the <auto run> key and press <4> RP (this represents the Reset or Print option). Selecting <1> will reset previous auto run sequences and <2> will print the run log. Press <auto run> until in the standby mode.

Next, enter sample ID for each tray position. Press <auto run> and a 1 will appear in the left side of the display indicating that position 1 on the auto run is being entered. Select <3> for sample (or 1 for blank, or 2 for k-factor) and enter the sample ID and hit <enter>. The instrument should advance to position 2. When finished entering the entire run, press <auto run> until the instrument is in the standby mode.

After entering the run, print the run set-up by following the instructions in the previous step.

When finished, press <auto run> again until the instrument says standby.

Place the auto sampling tray on top of the CHN making sure that the tray clicks into place so the advance arm is seated into position. Position 1 should be left empty when filling the sampling tray as there is a hole in it for the sample to drop into the instrument. After the tray is seated, place the 1st sample in position one. It should drop down into the instrument.

To start the run, press <start> and the sequence will begin. Watch to make sure that the sampling tray advances to the next position. If not seated correctly, the tray will run sample position 1 continuously.

Packing New Columns

When packing columns follow the diagram in the CHN manual or that is in the drawer next to the hood. Be sure to pack the combustion column by packing with a pipette.

When packing the reduction column, use a funnel to pour the copper reagent into the tube and use a shaker to pack the column tight. Shake column 3-4 times between adding the copper reagent.

Sample Preparation-PC/PN water filters

Flat tin disks are expensive, so be sure to use only one per sample. They can stick together. Also, the instrument blanks are based on 1 tin disk, so if two are stuck together, it will alter the result.

Use forceps to gently tap the edges of the tin disks, this will separate them.

The CT DEEP will fold filters in ½ in the field. They must be baked in the oven overnight at 105°C. Filters may be baked in the tin foil pouches that they were submitted in, but the pouch must be opened before being placed in the oven. After filters are cooked, close tin foil pouch and place in desiccator until ready for preparation.

After cooked, using forceps and gloves fold filter in ¼ and place point at edge of tin disk. Be sure to use a clean working platform. Fold tin and filter, rolling filter in tin disk so that no portion of the filter is showing. Fold each end of rolled tin and place in the pellet press. The press has a removable metal cylinder that if placed with the ridged edge up, will compact the filter, when flipped over with ridge down, it will pop out the filter after pressed. The height of the platform may be adjusted by turning clock-wise to raise the platform up. Ensure that a tightly compacted pellet comes out of the press by raising the platform throughout preparation as necessary.

Record sample tray positions in the CHN sample preparation logbook.

When running filters, it is necessary to run a blank filter. These have been prepped according to the LISS methods, which are to muffle the filters for 1 hour at 500°C before giving them to the CT DEEP to bring to the field. A batch is set aside during this preparation for instrument analysis and to verify the filters are blank before giving them to the CT DEEP. These filters are run every 10 samples and are prepped the same way. They are called an ID BLK because when entering the

sample ID as the numeric-alpha related code, the screen says ID BLK. A tin disk blank will have a sample ID of simply “blank”.

K-factors are still entered with weight values when running filters.

K-factor Preparation

When prepping the K-factor it is very important to be extremely careful not to drop any sample out of the tin disk. It is also important to make sure 1 disk is used per sample.

First fold tin disk into a cone and then place cone on balance platform and zero.

Next, take tin and place ~2mg of K-factor in bottom of cone and weigh, and record weight in logbook.

After weighing, be sure to take cone out of balance without dropping any sample. Fold up tin with sample enclosed in tin disk, being careful not to lose any sample. **IF SAMPLE IS LOST, IT WILL ALTER THE RESULT GREATLY!!!**

K-factors are run as a separate QC and when run, are entered into the instrument as a sample.

- The recovery values for the QC are:
    - *C = 71.09%*
    - *H = 6.71%*
    - *N = 10.36*

Printing Data

Highlight the month folder and click <file> <Print>. Select <print the entire results table> then <report using template>. Under template, select <detailed results> and then <save to file>.

Save the .RTF file on a flash drive and go to another computer.

Open a word document, adjust the paper size to 8.5x11 for the whole document then print. Note: The report prints backwards.

Shutting Down the Instrument

First, turn off the furnace by pressing <parameters> <12> then <enter>. Press number <2> to turn the furnace off. Press <12> and make sure that the number 2 is blinking, indicating the furnace is indeed, off. Press <parameters> until the instrument is in standby.

Next, press <monitor> <No> to not print the temperatures, and <1> <enter> so that the decrease in temperature can been watched.

Once the instrument has cooled to about 100°C, press <monitor> until the instrument says standby.

Next, press <diagnostics> <2> (for gas), <2> (for valves), and enter code <4> <enter> and turn the valve on by pressing <1>.

Press code <5> <enter> and <1> to turn on valve 5. Let stand for a count of 10 seconds and then press diagnostics until in standby mode.

Turn off the instrument and shut off all of the gasses.

Quality Control

- PN/PC quality control checks:
    - One tin disk blank is run per 10 samples.
    - One blank filter is run per 10 samples.
    - One k-factor is run per 10 samples.

Nitrate and Nitrite EPA 353.2

Scope and Application

EPA Method 353.2 is the reference method for measuring nitrate + nitrite in seawater by automated colorimetric determination. This section provides a stepwise procedure for bench use by laboratory personnel.

This method (Lachat method number 31-107-04-1-A) was developed for the quantitative analysis of nitrates in water and seawater. The applicable range is 0 to 0.5mg/L as nitrogen. Samples higher in range may be diluted and re-run. The holding time for this analysis is 28 days.

This method is based on automated colorimetric determination and is restricted to the use by or under the supervision of analysts experienced in the use of auto analyzers.

This method may be used for analysis of NO_X-N (nitrate + nitrite) or nitrite alone. NO_x-N values are obtained by activating the cadmium column and calibrating with NO_3^- standards. Nitrite (NO_2^--N) is calibrated with NO_2^--N standards and the cadmium column is not activated. Reagents remain the same. The Nitrate (NO_3^--N) value is calculated by subtracting the Nitrite (NO_2^--N) value from the Nitrate + Nitrite (NO_x-N) value. In the LISS dissolved NO_X-N is analyzed and reported.

Summary of Method

The whole water sample is filtered through a 47mm GF/F filter in the field. The filtrate is frozen at –10°C or below until analysis can be completed (samples must not be preserved with mercuric chloride or thiosulfate, as these degrade the copper-cadmium column used in this analysis). Analysis is completed within 28 days from arrival date at the laboratory. Samples for nitrate + nitrite are analyzed using flow injection on a Lachat. Nitrate is reduced to nitrite at pH 7.5 in a copperized cadmium column. The nitrate reduced to nitrite, plus any free nitrite present, reacts under acidic conditions with sulfanilamide to form a diazo compound that couples with N-1-Naphthylethylenediamine dihydrochloride to form a reddish-purple azo dye that is measured at 520nm. For nitrite analyses the cadmium column is not used.

The Method Detection Limit (MDL) and Practical Quantitation Limit (PQL) for this analysis are determined yearly. For current MDL and PQL limits, see the chart entitled Method Detection Limit Determination.

This method is based on automated colorimetric determinations and is restricted to the use by, or under the supervision of analysts experienced in the use of auto analyzer equipment.

The instruments are calibrated with a minimum of a six-point curve (including the blank) at the time of analysis (obtained from AccuStandard). The calibration curve is then verified by an external quality control sample from Fisher, an independent supplier. Supplier guidelines are used for making up the quality control solutions, as well as for information on the true value and acceptable value range for the analytes being measured in each quality control sample.

An initial calibration check along with an initial calibration blank, demonstrate that the instrument is capable of acceptable performance at the beginning of the sample analysis. In order to ensure continuing acceptable performance, a continuing calibration check and continuing calibration blank are run every tenth sample. For every 10 samples, a laboratory spike analysis and a laboratory duplicate analysis are performed. At the end of the run a practical quantitation limit (PQL) is run for further quality control verification.

Interferences

Build up of suspended matter in the cadmium column will restrict flow. Look for a "jerking" action in one or several of the pump tube lines as evidence of such a blockage. Nitrate-nitrogen is, however, found in a soluble state, so pre-filtering of samples should be sufficient to keep lines clear.

Low results are possible for samples high in metals concentrations such as iron or copper. (1.0 g per liter) $Na_2EDTA \cdot 2H_2O$ can be added to the buffer to reduce this interference.

Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. Pre-extracting the sample with an organic solvent eliminates this interference.

References

31-107-04-1-A, August 19, 2003. Determination of Nitrate/Nitrite in Brackish or Seawater by Flow Injection Analysis. Lachat Applications Group, Lachat Instruments, Loveland CO.

EPA Method 353.2. Determination of Nitrate-Nitrite Nitrogen by Automated Colorimetry. Revision 2.0, August 1993. Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Standard Method 4500-N. Nitrogen. Page 4-99—4-123, 20th Edition, 1998, Standard Methods for the Examination of Water and Wastewater.

Associated SOP’s

Refer to “SOP” titled “Hazardous Waste SOP” for proper waste disposal.

Refer to the notebook titled “SOP’s” located in the nutrients laboratory.

Safety

Cadmium crystals are a known carcinogen; use caution when reactivating the cadmium for column repacking.

Reagents are hazardous and are made in the hood. Protective gear is worn when making reagents.

Ensure that waste lines from the detector are going to the proper hazardous waste jug located under the instrument.

Refer to the University of Connecticut’s Environmental Health and Safety chemical health and safety web page at:

http://www.ehs.uconn.edu/ppp/index.php

A hard copy of the Chemical Hygiene Plan can be found on the notebook rack located on the lab bench. Also refer to the Hazardous Waste SOP.

Materials

Procedure

Reagent Preparation

Unless otherwise specified, all chemicals should be ACS grade or equivalent. DI water refers to high quality reagent water, TYPE I or TYPE II as defined in ASTM Standards, Part 31, D1193-74. When making reagents, date the container in which the reagent is stored, initial it, and write out the entire name of the reagent and hazard warnings.

- Ammonium Chloride Buffer

We are currently using Fisher hydrochloric acid (catalog no. A144S-212), Fisher disodium EDTA (catalog no. S311-500), and Fisher ammonium hydroxide (catalog no. A669S-212).

Hydrochloric Acid (concentrated)	105mL
Ammonium Hydroxide	95mL
Disodium EDTA	1.0g

Be sure to make this reagent in the hood. Wear all protective gear! Add about 500mL DI water to a 1000mL volumetric flask. Carefully pour in 105mL concentrated hydrochloric acid, rinse graduated cylinder with DI, then, with a new graduated cylinder, pour in 95mL ammonium hydroxide. Again rinse graduated cylinder with DI. Add 1.0 g disodium EDTA, dissolve and dilute to the mark. Add stir bar to mix and then adjust the pH to 8.5 with 2 N HCl or 15N NaOH solution. Solution is stable for two months.

- Sulfanilimide Color Reagent

We are currently using Fisher sulfanilamide (catalog no. AC132855000), Acros NED (catalog no. AC42399-0250), and Fisher phosphoric acid (catalog no. A242-212).

Phosphoric Acid (85% soln. by wt.)	100mL
Sulfanilamide	40.0g
NED (N-(1-napthyl)ethylenediamine dihydrochloride	1.0g

To a 1L volumetric flask, add about 600mL DI water then add 100mL 85% phosphoric acid, 40g sulfanilamide and 1.0g NED. Dilute to the mark, insert and then mix with stir bar over medium heat. Store in a dark bottle and discard when the solution turns pink, roughly one month.

Cadmium-Copper Reduction Column

Pre-packed cadmium columns are available from Lachat/HACH (Lachat part/order no. 50237A). Instructions for repacking columns in the laboratory are at the end of the Nitrate/Nitrite SOP and can be made available upon request.

Standard Preparation

Working standards can be made by weight or volume. Standards are made using a 1000µg/mL stock certified from AccuStandard or another source different from the QC. Standards are made weekly.

Stock Standard, 10.0 mg/L N

AccuStandard Stock (NO_3^--N)	1mL
DI Water, q.s.	100mL final vol.

In a 100mL volumetric flask containing about 80mL of DI add 1mL of stock AccuStandard NO_3^--N standard. Dilute to 100mL with DI and mix thoroughly. Record the information in the stock standard logbook.

Working Standard Solutions

mL(g) 10mg/L Stock	mg/L N
5.0	0.500
3.0	0.300
1.0	0.100
0.5	0.050
0.25	0.025
0.1	0.010

Transfer aliquots of stock 10mg/L stock as noted above to individual 100mL volumetric flasks. Dilute to volume with DI water and mix thoroughly. Record the information in working standard logbook. Prepare fresh weekly.

A Nitrite (NO_2^--N) QC of the same concentration as the NO_3^--N QC must also be made when running with the cadmium column to ensure that the cadmium column is working efficiently. A 90% recovery is considered acceptable. A lower recovery than this indicates that the cadmium column must be replaced. QC is prepared fresh daily.

Sample Preparation

Sample turbidity is removed by filtration though a 47mm GF/F filter prior to analysis. Turbidity absorbing in the range of 550 nanometers (nm) will present a positive bias.

Preserve the sample by freezing at -10°C or below until the time of analysis. Sample containers are to be rinsed with 1:1 hydrochloric acid, followed by DI water and finally by an aliquot of the sample itself. Disposable plastic sample tubes are used during analysis to avoid contamination from improper washing of glass tubes.

Generally, 5mL of sample is spiked with 100µL of the 10ppm stock standard, yielding a spike concentration of 0.196ppm.

The quality control sample (QC) is made up in 100mL volumes per every 60 or so samples. Larger quantities are necessary if running large batches of samples. Generally, a QC concentration of 0.3ppm is used and is made fresh daily.

Instrumental Analysis

It is assumed that the user is basically familiar with the appearance and location of the various parts of the Lachat. It is also assumed that a method for running NO_x analyses has already been created, and that the user is familiar with basic system operations. For a physical description of the instrument and any related software information, see the section entitled “The Lachat Quick Chem 8500”.

Ensure that the correct size sample loop (150cm) is attached at the manifold valve between ports 1 and 4.

Ensure that the 520nm wavelength filter is in the detector.

Ensure that the sample line is attached to port 6 of the switching valve and that the reagents are all pumping properly.

General Analyzer Information

When using the cadmium column, check the efficiency of the column daily by analyzing equal concentrations of nitrate and nitrite standards. The efficiency should be >90%.

Introduce the ammonium chloride reagent into the chemistry manifold first, allow the system to flow for about a minute, and then introduce the sulfanilamide.

When using the cadmium column, ALWAYS ensure that the column is activated when ALL reagents are pumping through the system. Likewise, make sure the column is in the “off” position at the end of the run before taking reagent lines out of solution for the wash step.

Cadmium columns are purchased from Lachat instruments (CAT # 50237A), however cadmium may be regenerated in the laboratory according to Lachat publication WI#J20008. This publication is available upon request.

Calculations

- Percent recovery for the spike is determined using the following formula:

$$\%R = \frac{(A - B)}{C} \times 100$$

Where:

A = measured value in mg/L for the sample + spike
B = measured value in mg/L for the original sample
C = concentration of the spike in mg/L

- Relative percent difference for the duplicate is calculated by the following formula:

$$RPD = \frac{(A - B)}{[(A + B) \div 2]} \times 100$$

Where:

A = the value in mg/L for the first run of the sample
B = the value in mg/L for the second run of the sample

- To determine the column efficiency, use the following formula:

$$E = \frac{[NO_3^- - N]}{[NO_2^- - N]} x100$$

Where:

E = column efficiency
NO_3^--N = concentration of nitrate standard
NO_2^--N = concentration of nitrite standard

Quality Control

A certified second source quality control sample (purchased from Fisher) is analyzed for every delivery group (or every 10 samples) and the value must be within 85-115% recovery to be considered acceptable.

A spike is analyzed for every delivery group (or every 10 samples). The percent recovery must fall within the 85-115% recovery to be acceptable.

A duplicate is analyzed for every delivery group (or every 10 samples) and the relative percent difference must be < 20%.

A blank is analyzed for every delivery group or every 10 samples and the value must fall below the practical quantitation limit (PQL) to be considered acceptable. The concentration of the PQL is the low standard.

A second quality control sample is analyzed at the end of the run and is the concentration of the PQL, or the lowest standard. Acceptable ranges for the PQL are 50-150% recovery.

Cadmium column efficiency is analyzed with every calibration.

Other System Notes

Light interference filter: 520nm
Sample Loop Size: 150cm

Ammonia EPA 350.1

Scope and Application

EPA Method 350.1 is the reference method for measuring ammonia in seawater by automated colorimetric determination with phenate. This section provides a stepwise procedure for bench use by laboratory personnel.

This method (Lachat ammonia method number 31-107-06-1-B) was developed for the quantitative analysis of ammonia in water and seawater. The applicable range is 0 to 0.5mg/L of ammonia as nitrogen (NH_3-N). Seawater samples higher in range may be diluted and analyzed using the same method.

The Method Detection Limit (MDL) and Practical Quantitation Limit (PQL) for this analysis are determined yearly. For current MDL and PQL limits, see the chart entitled Method Detection Limit Determination.

Contamination of samples with ammonia is a problem of great concern. Ammonia is ubiquitous in the environment. Ammoniated floor strippers and waxes are strictly prohibited in the laboratory.

This method is based on automated colorimetric determinations and is restricted to the use by, or under the supervision of, analysts experienced in the use of auto analyzer equipment.

Summary of Method

The whole water sample is filtered through a 47mm GF/F glass-fiber filter in the field. The filtrate is then frozen at or below –10°C until analysis can be completed. Analysis is completed within 28 days from arrival date at the laboratory. Samples for ammonia are analyzed by an automated procedure on a Lachat, utilizing the Berthelot reaction.

Ammonia in the sample reacts with alkaline sodium phenate and then sodium hypochlorite to form indophenol blue. A solution of EDTA is added to the sample stream to eliminate the precipitation of the hydroxides of calcium and magnesium. Sodium nitroprusside is added to intensify the blue color.

The Lachat is calibrated with a minimum of a six-point curve (including the blank) at the time of analysis (purchased from AccuStandard). The calibration curve is then verified by an external quality control sample from Fisher, an independent supplier). Supplier guidelines are used for making up the quality control solutions, as well as for information on the true value and acceptable value range for the analytes being measured in each quality control sample.

An initial calibration check along with an initial calibration blank, demonstrate that the instrument is capable of acceptable performance at the beginning of the sample analysis. To ensure continuing acceptable performance a continuing calibration check and continuing calibration blank are run every tenth sample. For every 10 samples, a laboratory spike analysis and a laboratory duplicate analysis are performed.

Interferences

Calcium and magnesium ions could precipitate if present in sufficient concentration. EDTA is added to the sample stream to rectify this problem.

Color (as well as certain organic species) can cause interference.

Method interferences may be caused by contaminants in the reagent water, reagents, glassware and other sample processing apparatus that may bias analytical results.

References

31-107-06-1-B, August 2003. Determination of Ammonia in Brackish or Seawater by Flow Injection Analysis. Lachat Instruments, Loveland, CO.

EPA Method 350.1. Determination of Ammonia Nitrogen by Semi-Automated Colorimetry. Revision 2.0, August 1993. Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Standard Method 4500-NH3 G Ammonia by Automated Phenate. Page 4-103—4-112, 20th Edition, 1998, Standard Methods for the Examination of Water and Wastewater.

Associated SOP's

Refer to "SOP" titled "Hazardous Waste SOP" for proper waste disposal.

Refer to the notebook titled "SOP's" located in the nutrients laboratory.

Safety

Reagents are hazardous and are made in the hood. Protective gear is worn when making reagents.

Phenol is a known carcinogen and is hazardous. Use caution when making this reagent. There are special gloves in the Phenol cabinet. Be sure to wear them when using this reagent.

Ensure that waste lines from the Lachat are going to the proper hazardous waste jug located under the instrument.

Refer to the University of Connecticut's Environmental Health and Safety chemical health and safety web page at:

http://www.ehs.uconn.edu/ppp/index.php

A hard copy of the Chemical Hygiene Plan can be found on the notebook rack located on the lab bench. Also refer to the Hazardous Waste SOP.

Materials

Lachat QuickChem8500

Procedure

Reagent Preparation

Unless otherwise specified, all chemicals should be ACS grade or equivalent. DI water refers to high quality reagent water, TYPE I or TYPE II as defined in ASTM Standards, Part 31, D1193-74. When making reagents, date the container in which the reagent is stored, initial it, write out the entire name of the reagent and hazard class.

- Phenol

We are currently using Fisher Scientific phenol crystals (catalog no. A92-100) and Fisher sodium hydroxide (catalog no. S318-3).

CAUTION: Phenol is very poisonous, causes severe burn, and is rapidly absorbed into the skin. Wear gloves and safety glasses.

Phenol Crystals	83g
Sodium Hydroxide	32g
DI Water, q.s.	1000mL final vol.

In a volumetric flask, fill ¾ with deionized water and dissolve 32 g of sodium hydroxide in approximately 600mL of water, dissolve and cool under cold tap water, being sure not to introduce tap water into the volumetric flask. Add 83g of phenol crystals and dilute to one liter with DI water and mix thoroughly. Store the reagent in an amber poly bottle. This material is corrosive, and is stable for about one week or until brown.

- Sodium Hypochlorite Solution

We are currently using Fisher bleach (Cat # SS290-1) that contains 5.65% NaOCl and no additives.

Sodium Hypochlorite Solution, 5.65%(Clorox)	50mL
DI Water, q.s.	50mL

Dilute 50mL of bleach to 50mL with DI water and mix thoroughly. Prepare fresh daily.

- Sodium Nitroprusside

We are currently using Fisher sodium nitroferricyanide dehydrates, 99% (sodium nitroprusside, catalog no. S350-100).

Sodium Nitroprusside	1.75g
DI water, q.s.	1000mL final vol.

Fill a volumetric flask ¾ with deionized water and add 1.75g of sodium nitroprusside in 1000mL of water and mix thoroughly. Store the solution in an amber poly bottle. Degas with helium for 5 minutes. Solution is stable for one week.

- Buffer Chelating Reagent

We are currently using Fisher EDTA (catalog no. S311-500) and Fisher sodium hydroxide (S399-212).

EDTA	50g
Sodium Hydroxide	11g
DI Water, q.s.	1000mL final vol.

In a 1L volumetric flask, fill ¾ with DI water, and add 50g of EDTA and 11g sodium hydroxide. Dilute to one liter and mix well. Store the solution in a clear poly bottle. Degas for 5 minutes with helium. Solution is stable for one week.

Standard Preparation

Working standards can be made by weight or volume. Standards are made using a 1000µg/mL stock certified from AccuStandard or another source different from the QC.

- Stock Standard, 10.0mg/L N

AccuStandard Stock (Ammonium NH_4-N)	1mL
DI water, q.s.	100mL final vol.

In a 100mL volumetric flask containing about 80mL of DI add 1mL of stock AccuStandard ammonium standard. Dilute to 100mL with DI and mix thoroughly. Record the standard information in the stock standard logbook. Standards are made fresh weekly.

Working Standard Solutions:

mL(g) 10mg/L Stock	mg/L N
5.0	0.500
3.0	0.300
1.0	0.100
0.5	0.050
0.25	0.025
0.1	0.010

Transfer aliquots of stock 10mg/L stock as noted above to individual 100mL volumetric flasks. Dilute to volume with DI water and mix thoroughly. Record the information in working standard logbook. Prepare fresh weekly.

Sample Preparation

Sample turbidity is removed by filtration though a 47mm GF/F filter prior to analysis. Turbidity absorbance in the range of 660 nanometers (nm) will present a positive bias.

Preserve the filtrate by freezing at –10°C or below until the time of analysis. Sample containers are to be rinsed with 1:1 hydrochloric acid, followed by DI water and finally by an aliquot of the sample itself. Disposable sample tubes are used during analysis to avoid contamination from improper washing of glass tubes.

Generally, 5mL of sample is spiked with 100µL of the 10ppm stock standard, yielding a spike concentration of 0.196ppm.

The quality control sample (QC) is made up in 100mL volumes per every 60 or so samples. Larger quantities are necessary if running large batches of samples. Generally, the concentration of the QC is 0.3ppm and is prepared fresh daily.

Instrumental Analysis

It is assumed that the user is basically familiar with the appearance and location of the various parts of the Lachat QuickChem Autoanalyzer. It is also assumed that a method for running ammonia analysis has already been created, and that the user is familiar with basic system operations. For a physical description of the instrument and any related software information, see the section entitled "The Lachat Quick Chem 8500".

The pH of the final reaction solution must lie within certain limits. Collect the solution from the flow cell waste line to verify the pH is between 11.5 and 11.9 if issues arise.

It is very important to introduce the reagent lines in this order: buffer, phenol, nitroprusside then bleach. When removing reagent lines when shutting down the instrument, do so in reverse order to prevent calcium precipitate from forming on the inside of the coils.

Ensure that the pump tubes are pumping reagents and that the sample line is hooked up to port 6 of the switching valve.

Calculations

- Percent recovery for the spike is determined using the following formula:

$$\%\mathrm{R} = \frac{(A - B)}{C} \times 100$$

Where:

A = measured value in mg/L for the sample + spike
B = measured value in mg/L for the original sample
C = concentration of the spike in mg/L

- Relative percent difference for the duplicate is calculated by the following formula:

$$\mathrm{RPD} = \frac{(A - B)}{[(A + B) \div 2]} \times 100$$

Where:

A = the value in mg/L for the first run of the sample
B = the value in mg/L for the second run of the sample

Quality Control

A certified second source quality control sample (purchased from Fisher) is analyzed for every delivery group (or every 10 samples) and the value must be within 85-115% recovery to be considered acceptable.

A spike is analyzed for every delivery group (or every 10 samples). The percent recovery must fall within the 85-115% recovery to be considered acceptable.

A duplicate is analyzed for every delivery group (or every 10 samples). The duplicate relative percent difference (RPD) must be below 20%.

A blank is analyzed for every delivery group or every 10 samples and the value must fall below the practical quantitation limit (PQL) to be considered acceptable. The concentration of the PQL is the low standard.

A second quality control sample is analyzed at the end of the run and is the concentration of the PQL, or the lowest standard. Acceptable ranges for the PQL are 50-150% recovery.

Other System Notes

Chemistry Manifold 1
Light interference filter: 660 nm
Special instructions: The reaction module for ammonia determinations comes equipped with a heating coil that heats the sample stream (after the addition of the reagents) to 37°C, which promotes better color development. This coil should be given 15 minutes to warm up before any samples are run.

Dissolved Inorganic Phosphorus EPA 365.1

Scope and Application

EPA Method 365.1 (Aspila, EPA) is the reference method for measuring dissolved inorganic phosphorus (DIP) in seawater by automated colorimetric determination. This section provides a stepwise procedure for bench use by laboratory personnel.

This method (Lachat ortho-phosphorus method number 31-115-01-1-H) was developed for the quantitative analysis of DIP in seawater. The applicable range is 0.010 to 0.500mg/L of DIP. Samples higher in range may be diluted and re-run or analyzed by recalibrating with higher concentration.

This method is based on automated colorimetric determination and is restricted to the use by or under the supervision of analysts experienced in the use of auto analyzers.

The holding time for this method is 28 days.

Summary of Method

For dissolved inorganic phosphorus, the whole water sample is filtered though a 47mm GF/F filter in the field. The filtrate is then preserved by freezing at or below -10°C until analysis is completed within 28 days. Samples for DIP are analyzed by an automated procedure on the Lachat QuickChem flow injection analyzer. The analysis depends on the formation of a phosphomolybdenum blue complex, which is read colorimetrically at 880 nm.

The Method Detection Limit (MDL) and Practical Quantitation Limit (PQL) for this analysis are determined yearly. For current MDL and PQL limits, see the chart entitled Method Detection Limit Determination.

This method is based on automated colorimetric determinations and is restricted to the use by, or under the supervision of, analysts experienced in the use of auto analyzer equipment.

The Lachat is calibrated with a minimum of a six-point curve (including the blank) at the time of analysis (obtained from AccuStandard). The calibration curve is then verified by an external quality control sample from Fisher, an independent supplier. Supplier guidelines are used for making up the quality control solutions, as well as for information on the true value and acceptable value range for the analytes being measured in each quality control sample.

An initial calibration check along with an initial calibration blank, demonstrate that the instrument is capable of acceptable performance at the beginning of the sample analysis. In order to ensure continuing acceptable performance a continuing calibration check and continuing calibration blank are run every tenth sample. For every 10 samples, a laboratory spike analysis and a laboratory duplicate analysis are performed. At the end of the run a practical quantitation limit (PQL) is run for further quality control verification.

Interferences

Arsenate is analyzed similarly to ortho-phosphorus and will cause interference if present. Reducing the arsenic acid to arsenious acid with sodium bisulfite should alleviate this problem. Sodium bisulfite treatment will also take care of any problems with high iron concentration (>50mg/L).

Any silica present will react with the reagents in this method, forming a pale blue complex which also absorbs at 880nm. Because the method is very sensitive to small amounts of phosphorus, sensitivity to silica is also high. Glass should therefore be avoided if possible. Reagents should be made and stored in plastic. Silica forms a pale blue complex that also absorbs at 880nm and is generally insignificant because a silica concentration of approximately 30mg/L would be required to produce a 0.005 P/L positive error in orthophosphate.

Acidity among samples, standards and blanks should be carefully controlled. Large variations in acidity will affect sample and/or standard peaks.

Good glassware cleaning procedures should always be used. Phosphorus contamination is a constant problem. Proper glassware washing protocol should elevate this problem.

References

31-115-01-1-H, August 2003. Determination of Orthophosphate by Flow Injection Analysis. Lachat Instruments, Loveland, CO.

EPA Method 365.1. Determination of Phosphorus by Semi-Automated Colorimetry. Revision 2.0, August 1993. Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Standard Method 4500-P A, B, G and H Phosphorous: Flow Injection Method. Page 4-139 – 4-153, 20th Edition, 1998, Standard Methods for the Examination of Water and Wastewater.

Associated SOP’s

Refer to “SOP” titled “Hazardous Waste SOP” for proper waste disposal.

Refer to the notebook titled “SOP’s” located in the nutrients laboratory.

Safety

Reagents are hazardous and are made in the hood. Protective gear is worn when making reagents.

Ensure that waste lines from the Lachat are going to the proper hazardous waste jug located under the instrument.

Refer to the University of Connecticut’s Environmental Health and Safety chemical health and safety web page at:

http://www.ehs.uconn.edu/ppp/index.php

A hard copy of the Chemical Hygiene Plan can be found on the notebook rack located on the lab bench. Also refer to the Hazardous Waste SOP.

Materials

Lachat QuickChem Auto Analyzer

Procedure

Reagent Preparation

Unless otherwise specified, all chemicals should be ACS grade or equivalent. DI water refers to high quality reagent water, TYPE I or TYPE II as defined in ASTM Standards, Part 31, D1193-74. When making reagents, date the container in which the reagent is stored, initial it, and write out the entire name of the reagent and hazard class.

- Stock Antimony Potassium Tartrate

We are currently using Fisher antimony potassium tartrate (catalog no. A867-250).

Antimony Potassium Tartrate	1.61g
DI Water, q.s.	500mL final vol.

Dissolve 1.61 g of antimony potassium tartrate in about 400mL of DI water in a 500mL volumetric flask. Dilute to 100mL with DI water and mix thoroughly. Store the solution in a dark plastic container. Solution is stable for one month.

- 1N Sulfuric Acid

We are currently using Fisher sulfuric acid (catalog no. SA176-4).

Sulfuric Acid	28mL
DI Water, q.s.	1000mL final vol.

Fill 1000mL volumetric flask ¾ with DI water and add 28mL of sulfuric acid. Dilute to 1000mL with DI water and mix thoroughly. Solution is stable for one month.

- Stock Ammonium Molybdate

We are currently using Fisher ammonium molybdate (catalog no. A674-500).

Ammonium Molybdate	20g
DI Water, q.s.	500mL final vol.

Fill 500mL volumetric flask ¾ with DI water and add 20g of ammonium molybdate. Dilute to final volume with DI water and mix thoroughly. Solution is stable for one month.
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- Molybdate Color Reagent

We are currently using Fisher Sulfuric Acid (catalog no. SA176-4).

Stock Antimony Potassium Tartrate Solution	72mL
Stock Ammonium Molybdate	213mL
Sulfuric Acid	35mL
DI Water, q.s.	1000mL final vol.

To a 1000mL volumetric flask add about 500mL of DI water, then 35mL of concentrated sulfuric acid. Swirl to mix. Add 213mL of stock ammonium molybdate solution and 72mL of stock antimony potassium tartrate solution. Dilute to 1000mL with DI water and mix thoroughly. Degas with helium for at least 5 minutes. Store in a amber plastic container. This solution is stable for one month.

- Ascorbic Acid

We are currently using Fisher, L-ascorbic acid (catalog no. BP351-500) and Fisher SDS (catalog no. BP166-100).

Ascorbic Acid	60g
SDS	1g
DI Water, q.s.	1000mL final vol.

In a 1L volumetric add 60g of ascorbic acid in approximately 800mL DI water. Dilute to 1000 mL with DI water and mix thoroughly. Degas for a minimum of 5 minutes. Pour into clear plastic bottle and add 1g of SDS and swirl gently. This solution is stable for 5 days. Store the solution in a clear poly container.

Standard Preparation

Working standards can be made by weight or volume. Standards are made using a 1000ug/mL stock certified from AccuStandard or another source different from the QC.

- Stock Standard, 10.0mg/L N

AccuStandard Stock (Phosphorus)	1mL
DI Water, q.s.	100mL

In a 100mL volumetric flask containing about 80mL of DI add 1mL of stock AccuStandard phosphorus standard. Dilute to 100mL with DI and mix thoroughly. Record the information in the stock standard logbook. Prepare fresh weekly.

Working Standard Solutions:

mL(g) 10mg/L Stock	mg/L P
5.0	0.500
3.0	0.300
1.0	0.100
0.5	0.050
0.25	0.025
0.1	0.010

Transfer aliquots of stock 10mg/L stock as noted above to individual 100mL volumetric flasks. Dilute to volume with DI water and mix thoroughly. Record the information in working standard logbook. Prepare fresh weekly.

Sample Preparation

Turbidity absorbing in the range of 880 nanometers (nm) will present a positive bias.

Preserve the sample by freezing at -10°C or below until the time of analysis. Sample containers are to be rinsed with 1:1 hydrochloric acid, followed by DI water and finally by an aliquot of the sample itself.

Generally, 5mL of sample is spiked with 100µL of the 10ppm stock standard, yielding a spike concentration of 0.196ppm.

The quality control sample (QC) is made up in 100mL volumes per every 60 or so samples. Larger quantities are necessary if running large batches of samples. Generally, the QC concentration is 0.3ppm and is prepared fresh daily.

Instrumental Analysis

It is assumed that the user is basically familiar with the appearance and location of the various parts of the Lachat. It is also assumed that a method for running ortho-phosphorus analysis has already been created, and that the user is familiar with basic system operations. For a physical description of the instrument and any related software information, see the section entitled “The Lachat Quick Chem 8500”.

Ensure that reagents are pumping through all pump tubes and that the sample line is connected to port 6 of the switching valve.

Ensure that the proper method has been selected, either for fresh water analysis or one that utilizes the refractive index correction for seawater samples.

Calculations

- Percent recovery for the spike is determined using the following formula:

$$\%\mathrm{R} = \frac{(A - B)}{C} \times 100$$

Where:

A = measured value in mg/L for the sample + spike
B = measured value in mg/L for the original sample
C = concentration of the spike in mg/L

- Relative percent difference for the duplicate is calculated by the following formula:

$$\mathrm{RPD} = \frac{(A - B)}{[(A + B) \div 2]} \times 100$$

Where:

A = the value in mg/L for the first run of the sample
B = the value in mg/L for the second run of the sample

Quality Control

A certified second source quality control sample (purchased from Fisher) is analyzed for every delivery group (or every 10 samples) and the value must be within 85-115% recovery to be considered acceptable.

A spike is analyzed for every delivery group (or every 10 samples). The percent recovery must fall within the 85-115% recovery to be acceptable.

A duplicate is analyzed for every delivery group (or every 10 samples) and the relative percent difference must fall below 20%.

A blank is analyzed for every delivery group or every 10 samples and the value must fall below the practical quantitation limit (PQL) to be considered acceptable. The concentration of the PQL is the low standard.

A second quality control sample is analyzed at the end of the run and is the concentration of the PQL, or the lowest standard. Acceptable ranges for the PQL are 50-150% recovery.

Other System Notes

Chemistry manifold 2
Light interference filter: 880 nm
Special instructions: The reaction module for phosphorus determinations comes equipped with a heating coil that heats the sample stream (after the addition of the reagents) to 37°C, which promotes better color development. This coil should be given 15 minutes to warm up before any samples are run.

Total Dissolved Nitrogen EPA 353.2

Scope and Application

This is an alkaline persulfate oxidation method (D'Elia 1977) on seawater for total dissolved nitrogen (TDN). Nitrate is the sole N product of the digestion and is determined by an automated colorimetric procedure. This section provides a stepwise procedure for bench use by laboratory personnel.

EPA Method 353.2 is the reference method for measuring nitrate + nitrite in seawater by automated colorimetric determination, and SM 4500 N C is the digestion. This section provides a stepwise procedure for bench use by laboratory personnel.

This method is based on automated colorimetric determination and is restricted to the use by or under the supervision of analysts experienced in the use of auto analyzers. The applicable range is from 0.0—2.0mg/L. The holding time for this analysis is 28 days.

Samples are extracted with potassium persulfate and N values are obtained by activating the cadmium column and calibrating with combined NO_3-N + NH_4-N standards for the TDN calibration curve.

Summary of Method

For total dissolved nitrogen (TDN) analysis, the whole water sample is filtered though a 47mm GF/F filter in the field. 10mL of sample is then pipetted into a screw cap test tube. The pipetted sample is then frozen at –10°C or below until digestion can be completed. 5mL of an oxidizing reagent (potassium persulfate) is then added. The tubes are placed in an autoclave at 235°F for 60 minutes. The sample is allowed to sit overnight and then is ready for analysis of TDN. Analysis is completed within 28 days of arrival at the laboratory.

Every 10 samples, a preparation blank, a laboratory spike and a laboratory duplicate analysis are performed. Samples are analyzed using flow injection on the Lachat. Nitrate is reduced to nitrite at pH 7.5 in a copperized cadmium column. The nitrate reduced to nitrite, plus any free nitrite present, reacts under acidic conditions with sulfanilamide to form a diazo compound that couples with N-1-Naphthylethylenediamine dihydrochloride to form a reddish-purple azo dye that is measured at 550nm.

The Method Detection Limit (MDL) and Practical Quantitation Limit (PQL) for this analysis are determined yearly. For current MDL and PQL limits, see the chart entitled Method Detection Limit Determination.

This method is based on automated colorimetric determinations and is restricted to the use by, or under the supervision of, analysts experienced in the use of auto analyzer equipment.

The analyzer is calibrated with a minimum of a six-point curve (including the blank) at the time of analysis (obtained from AccuStandard). The calibration curve is then verified by an external quality control sample from Fisher, an independent supplier. Supplier guidelines are used for making up the quality control solutions, as well as for information on the true value and acceptable value range for the analytes being measured in each quality control sample.

Initial calibration verification, along with an initial calibration blank, demonstrates that the instrument is capable of acceptable performance at the beginning of the sample analysis. To ensure continuing acceptable performance a continuing calibration check and continuing calibration blank are run every tenth sample. For every 10 samples, a laboratory spike analysis and a laboratory duplicate analysis are performed. At the end of the run a practical quantitative limit (PQL) is run for further quality control verification.

Interferences

Build up of suspended matter in the cadmium column will restrict flow. Look for a "jerking" action in one or several of the pump tube lines as evidence of such a blockage. Nitrate nitrogen is, however, found in a soluble state, so pre-filtering of samples should be sufficient to keep lines clear.

Low results are possible for samples high in metals concentrations such as iron or copper. (1.0g per liter) $Na_2EDTA \cdot 2H_2O$ can be added to the buffer to reduce this interference.

Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. Pre-extracting the sample with an organic solvent eliminates this interference.

References

10-107-04-1-C, March 2003. NitrateDetermination of Nitrate/Nitrite in Surface and Wastewaters by Flow Injection Analysis. Lachat Instruments, Loveland, CO.

31-107-044-A, September 18, 2003. Determination of Total Nitrogen in Brackish or Seawater by Flow Injection Analysis. Lachat Instruments Applications Group, Loveland, CO.

EPA Method 353.2. Determination of Nitrate-Nitrite Nitrogen by Automated Colorimetry. Revision 2.0, August 1993. Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Standard Method 4500 –N C. Nitrogen: Persulfate Method. Page 4-102—4-103, 20th Edition, 1998, Standard Methods for the Examination of Water and Wastewater.

Associated SOP's

Refer to "SOP" titled "Hazardous Waste SOP" for proper waste disposal.

Refer to the notebook titled "SOP's" located in the nutrients laboratory.

Safety

Samples are disposed of in a hazardous waste jug and are properly labeled.

Reagents are hazardous and are made in the hood. Protective gear is worn when making reagents.

Ensure that waste lines from the Lachat are going to the proper hazardous waste jug located under the instrument.

Refer to the University of Connecticut's Environmental Health and Safety chemical Health and safety web page at:

http://www.ehs.uconn.edu/ppp/index.php

A hard copy of the Chemical Hygiene Plan can be found on the notebook rack located on the lab bench. Also refer to the Hazardous Waste SOP.

Materials

Market Forge Autoclave
Lachat Quick Chem 8500
Cadmium column

Procedure

Reagent Preparation

Unless otherwise specified, all chemicals should be ACS grade or equivalent. DI water refers to high quality reagent water, TYPE I or TYPE II as defined in ASTM Standards, Part 31, D1193-74. When making reagents, date the container in which the reagent is stored, initial it, and write out the entire name of the reagent.

- Digestion Reagent – Potassium Persulfate

We are currently using Fisher sodium hydroxide (catalog no.S318-3) and Fisher potassium persulfate (catalog no. P282-500). The potassium persulfate should be kept in a desiccator to minimize the possibility of oxidization.

Sodium Hydroxide	6.0g
Potassium Persulfate	40.2g
Boric Acid	12.0g
DI Water, q.s.	1000mL final vol.

In a 1000mL volumetric flask, dissolve 6.0g sodium hydroxide in about 600mL of water. When the sodium hydroxide is completely dissolved, add 40.2g potassium persulfate and 12.0g of boric acid and dissolve with a magnetic stirrer. Dilute to 1 liter with DI water and mix thoroughly. This solution is unstable and should be prepared immediately prior to use.

- Ammonium Chloride Buffer

We are currently using Fisher hydrochloric acid (catalog no. S318-3) and Fisher ammonium hydroxide (catalog no. A669S-212).

Hydrochloric Acid (concentrated)	210mL
Ammonium Hydroxide	190mL
Disodium EDTA	2.0g

Be sure to make this reagent in the hood. Wear all protective gear! Add about 500mL DI water to a 1000mL glass volumetric flask. Carefully pour in 210mL concentrated hydrochloric acid and rinse well. With a new graduated cylinder, pour in 190mL ammonium hydroxide and rinse. Add 1.0g disodium EDTA, dissolve and dilute to the mark. Add stir bar and mix then adjust the pH to 8.5 with 15N sodium hydroxide solution. Solution is stable for two months.

- Sulfanilamide Color Reagent

We are currently using Fisher sulfanilamide (catalog no. O4525-100), Acros NED (catalog no. AC42399-0250), and Fisher phosphoric acid (catalog no. A242SK-2212).

Phosphoric Acid (85% soln. by wt.)	200mL
Sulfanilamide	80.0g
NED (N-(1-napthyl)ethylenediamine dihydrochloride	2.0g

To a 1000mL volumetric flask, add about 600mL DI water then add 200mL 85% phosphoric acid, 80g sulfanilamide and 2.0g NED. Dilute to final volume, add stir bar and heat over medium heat to dissolve. Store in a dark bottle and discard when the solution turns pink, roughly one month.

- 2N Hydrochloric Acid

We are currently using Fisher hydrochloric acid (catalog no. S318-3).

Hydrochloric Acid (concentrated)	16.6mL
DI Water, q.s.	100mLfinal vol.

Add 50mL DI water to a graduated cylinder. Pour in carefully 16.6mL hydrochloric acid and dilute to 100mL with DI water. Prepare fresh quarterly.

Cadmium-Copper Reduction Column

Pre-packed cadmium columns are used with the Lachat nitrate/nitrite manifold are available from Lachat/HACH (Lachat part/order no. 50237A).

Standard Preparation

Working standards can be made by weight or volume. Standards are made using a 1000µg/mL stock certified from AccuStandard or another source different from the QC. Solutions are stable for one week.

Stock Standard, 20.0mg/L N

AccuStandard Stock (NH_4 and NO_3 as N)	1mL of each
DI water, q.s.	100mL

In a 100mL volumetric flask containing about 80mL of DI add 1mL each of stock AccuStandard NH_3-N and NO_3^--N standards. Dilute to 100mL with DI and mix thoroughly. Record the information in the stock standard logbook.

Working Standard Solutions

mL(g) 20mg/L Stock	mg/L N
10	2.0
5	1.0
3	0.6
1	0.2
0.05	0.1
0.025	0.05

Transfer aliquots of stock 20mg/L stock as noted above to individual 100mL volumetric flasks. Dilute to volume with DI water and mix thoroughly. Record the information in working standard logbook. Prepare fresh weekly.

Cadmium column efficiency is not tested for this analysis because the calibrants are digested in potassium persulfate. Ensure that column efficiency has been tested prior to this run on the most recent NO_x analysis and has fallen within acceptable limits.

Sample Preparation

Sample turbidity is removed by filtration though a 47mm GF/F prior to analysis and will yield the TDN result. Turbidity absorbing in the range of 550 nanometers (nm) will present a positive bias.

Preserve the sample by freezing at -10°C or below until the time of analysis.

Sample containers are to be rinsed with 1:1 hydrochloric acid, followed by DI water and finally by an aliquot of the sample itself. Disposable sample tubes are used during analysis to avoid contamination from improper washing of glass tubes.

Pipette 10mL of sample into a 30mL test tube. 10mL of standards, QC and blanks should also be pipetted. The lowest concentration of standard is pipetted 6 times to allow for running the PQL throughout the run. The rest of the standards are pipetted at least 3 times for each concentration.

The quality control sample (QC) is made up in 100mL volumes per every 60 or so samples. Larger quantities are necessary if running large batches of samples. The QC concentration changes with each new lot number purchased from Environmental Resource Associates and is prepared fresh daily.

Generally, the sample is spiked with 250µL of the 20ppm stock standard and are spiked directly into the test tube before digestion, yielding a spike concentration of 0.488ppm.

Add 5mL of digestion reagent and mix thoroughly for a final volume of 15mL in the test tube. Place the samples and standards into the autoclave and heat from 235°F for one hour.

Allow the autoclave pressure to equalize, and the temperature to decrease removing the sample. Cool to room temperature overnight.

If analysis cannot be performed immediately samples can be stored at 4°C after digestion.

Instrumental Analysis

Transfer the samples to disposable test tubes for automated TDN analysis on the Lachat (method 31-107-04-4-A).

It is assumed that the user is basically familiar with the appearance and location of the various parts of the Lachat. It is also assumed that a method for running TDN analyses has already been created, and that the user is familiar with basic system operations. For a physical description of the instrument and any related software information, see the section entitled "The Lachat Quick Chem 8500".

Ensure that the proper sized sample loop is connected between ports 1 and 4 of the inject valve.

The sample loop is 150cm long and labeled "TN". It can be found in the drawer of Lachat parts.

The column efficiency should be greater than 90%. When the efficiency falls outside of this range, the cadmium column must be replaced.

Introduce the ammonium chloride reagent into the chemistry manifold first and let it flow for about a minute before introducing the sulfanilamide.

When using the cadmium column, ALWAYS ensure that the column is activated when ALL reagents are pumping through the system. Likewise, make sure the column is in the "off" position at the end of the run before taking reagent lines out of solution for the wash step.

Cadmium columns are purchased from Lachat instruments (CAT # 50237A), however cadmium may be regenerated in the laboratory according to Lachat publication WI#J20008. Publication will be made available upon request.

Calculations

- Percent recovery for the spike is determined using the following formula:

$$\%R = \frac{(A - B)}{C} \times 100$$

Where:

A = measured value in mg/L for the sample + spike
B = measured value in mg/L for the original sample
C = concentration of the spike in mg/L

- Relative percent difference for the duplicate is calculated by the following formula:

$$\mathrm{RPD} = \frac{(A - B)}{[(A + B) \div 2]} \times 100$$

Where: A = the value in mg/L for the first run of the sample
B = the value in mg/L for the second run of the sample

- To determine the column efficiency use the following formula:

$$\mathrm{E} = \frac{[\mathrm{NO_3^-} \text{ - N}]}{[\mathrm{NO_2^-} - \mathrm{N}]} \times 100$$

Where:

E = column efficiency
NO_3^--N = concentration of nitrate standard
NO_2^--N = concentration of nitrite standard

Quality Control

A certified second source quality control sample (purchased from Fisher) is analyzed for every delivery group (or every 10 samples) and the value must be within 85-115% recovery to be acceptable.

A spike is analyzed for every delivery group (or every 10 samples). The percent recovery must fall within the 85-115% recovery to be acceptable.

A duplicate is analyzed for every delivery group (or every 10 samples). The relative percent difference for the duplicate analysis must fall below 20%.

A blank is analyzed for every delivery group or every 10 samples and the value must fall below the practical quantitation limit (PQL) to be considered acceptable. The concentration of the PQL is the low standard.

A second quality control sample is analyzed at the end of the run and is the concentration of the PQL, or the lowest standard. Acceptable ranges for the PQL are 50-150% recovery.

Other System Notes

Light interference filter: 520nm
Sample Loop Size: 150cm

Total Dissolved Phosphorus EPA 365.1

Scope and Application

EPA Method 365.1 is the reference method for the measurement of total dissolved phosphorus (TDP) in seawater after preliminary digestion with sodium persulfate.

The Method Detection Limit (MDL) and Practical Quantitation Limit (PQL) for this analysis are determined yearly. For current MDL and PQL limits, see the chart entitled Method Detection Limit Determination.

This section provides a stepwise procedure for bench use by laboratory personnel.

This method (Lachat method number 31-115-01-1-H) was developed for the quantitative analysis of ortho-phosphate in water and seawater. The applicable range is 0 to 0.5mg/L of ortho-phosphate as phosphorus. Samples higher in range may be diluted and re-run or analyzed calibrating with a higher concentration (usually 1.0ppm).

This method is based on automated colorimetric determination and is restricted to the use by or under the supervision of analysts experienced in the use of auto analyzers.

Summary of Method

For total dissolved phosphorus, the whole water sample is filtered through a 0.7mm GF/F filter. The filtrate is then preserved by freezing at or below -10°C until it is time for preparation. The filtered sample will yield total dissolved phosphorus (TDP) values, while the whole water sample will yield total phosphorus (TP) values. Analysis is completed within 28 days of arrival at the laboratory.

The sample is digested with sodium persulfate in an autoclave at 235°F for one hour.

Samples for TDP are analyzed by an automated procedure on the Lachat flow analyzer. An aliquot of digested sample is reacted with reagents containing sulfuric acid, antimony tartrate, ammonium molybdate and ascorbic acid, and the resulting molybdenum blue complex is measured photometrically at 880nm.

This method is based on automated colorimetric determinations and is restricted to the use by, or under the supervision of, analysts experienced in the use of auto analyzer equipment.

The Lachat QuickChem is calibrated with a minimum of a six-point curve (including the blank) at the time of analysis (purchased from AccuStandard). The calibration curve is then verified by an external quality control sample from Fisher, an independent supplier. Supplier guidelines are used for making up the quality control solutions, as well as for information on the true value and acceptable value range for the analytes being measured in each quality control sample.

This initial calibration check along with an initial calibration blank, demonstrate that the instrument is capable of acceptable performance at the beginning of the sample analysis. In order to ensure continuing acceptable performance a continuing calibration check and continuing calibration blank are run every tenth sample. For every 10 samples, a laboratory spike analysis and a laboratory duplicate analysis are performed. At the end of the run a practical quantitative limit (PQL) is run for further quality control verification.

Interferences

Arsenate is analyzed similarly to orthophosphate and will cause interference if present. Reducing the arsenic acid to arsenious acid with sodium bisulfite should alleviate this problem. Sodium bisulfite treatment will also take care of any problems with high iron concentration (>50 mg/L).

Silica forms a pale blue complex that also absorbs at 880nm and is generally insignificant because a silica concentration of approximately 30mg/L would be required to produce a 0.005 P/L positive error in orthophosphate.

Acidity among samples, standards and blanks should be carefully controlled. Large variations in acidity will affect sample and/or standard peaks.

Good glassware cleaning procedures should always be used. Phosphorus contamination is a constant problem. Proper glassware washing protocol should eliminate this problem.

References

31-115-01-1-H, August 2003. Determination of Orthophosphate by Flow Injection Analysis. Lachat Instruments, Loveland, CO.

EPA Method 365.1. Determination of Phosphorus by Semi-Automated Colorimetry. Revision 2.0, August 1993. Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Standard Method 4500-P A, B, G and H Phosphorous: Flow Injection Method. Page 4-139 – 4-153, 20th Edition, 1998, Standard Methods for the Examination of Water and Wastewater.

Associated SOP's

Refer to "SOP" titled "Hazardous Waste SOP" for proper waste disposal.

Refer to the notebook titled "SOP's" located in the nutrients laboratory.

Safety

Samples are disposed in a hazardous waste jug and are appropriately labeled.

Reagents are hazardous and are made in the hood. Protective gear is worn when making reagents.

Ensure that waste lines from the Lachat are going to the proper hazardous waste jug located under the instrument.

Refer to the University of Connecticut's Environmental Health and Safety chemical Health and safety web page at:

http://www.ehs.uconn.edu/ppp/index.php

A hard copy of the Chemical Hygiene Plan can be found on the notebook rack located on the lab bench. Also refer to the Hazardous Waste SOP.

Materials

Lachat QuickChem 8500
Market Forge Autoclave

Procedure

Reagent Preparation

Unless otherwise specified, all chemicals should be ACS grade or equivalent. DI water refers to high quality reagent water, TYPE I or TYPE II as defined in ASTM Standards, Part 31, D1193-74. When making reagents, date the container in which the reagent is stored, initial it, and write out the entire name of the reagent.

- Stock Antimony Potassium Tartrate

We are currently using Fisher antimony potassium tartrate (catalog no. A867-250).

Antimony Potassium Tartrate	1.61g
DI Water, q.s.	500mL final vol.

Dissolve 1.61 g of antimony potassium tartrate in about 400mL of DI water in 500mL volumetric flask. Dilute to 100mL with DI water and mix thoroughly. Store the solution in a dark plastic container. Solution is stable for one month.

- Stock Ammonium Molybdate

We are currently using Fisher ammonium molybdate (catalog no. A674-500).

Ammonium Molybdate	20g
DI Water, q.s.	500mL final vol.

Fill 500mL volumetric flask ¾ with DI water and add 20g of ammonium molybdate. Dilute to final volume with DI water, mix thoroughly and store in amber poly bottle. Solution is stable for one month.

- Molybdate Color Reagent

We are currently using Fisher sulfuric acid (catalog no. SA176-4).

Stock Antimony Potassium Tartrate Solution	72mL
Stock Ammonium Molybdate	213mL
Sulfuric Acid	35mL
DI Water, q.s.	1000mL final vol.

To a 1-liter volumetric flask add about 500mL of DI water, then 35mL of concentrated sulfuric acid. Swirl to mix. Add 213mL of stock ammonium molybdate solution and 72mL of stock antimony potassium tartrate solution. Dilute to 1000mL with DI water and mix thoroughly. Degas with helium for at least 5 minutes. Store in a dark plastic container. This solution is stable for one month.

- Ascorbic Acid

We are currently using Fisher, L-ascorbic acid (catalog no. BP351-500) and Fisher SDS (catalog no. BP166-100).

Ascorbic Acid	60g
SDS	1g
DI Water, q.s.	1000mL final vol

In a 1L volumetric add 60g of ascorbic acid in approximately 800mL DI water. Dilute to 1000 mL with DI water and mix thoroughly. Degas for a minimum of 5 minutes. Pour into clear plastic bottle and add 1g of SDS and swirl gently. This solution is stable for 5 days. Store the solution in a clear poly container.

- Digestion Reagent -- Sodium Persulfate

We are currently using Fisher sodium persulfate (catalog no.AC20202-0010) and Fisher sulfuric acid (catalog no. SA176-4).

Sulfuric Acid	11.4mL
Sodium Persulfate	50g
DI Water, q.s.	1000mL final vol.

Add 11.4mL sulfuric acid in a 1L volumetric flask, mix and cool under tap water. Dissolve 50g of sodium persulfate and dilute to final volume of 1000mL with DI. The solution is not stable and should be prepared immediately prior to use. Store in a clear small mouth poly bottle.

Standard Preparation

Working standards can be made by weight or volume. Standards are made using a 1000µg/mL stock certified from AccuStandard or another source different from the QC and are prepared fresh weekly.

Stock Standard, 10.0mg/L N

AccuStandard Stock (Phosphorous)	1mL
DI water, q.s.	100mL

In a 100mL volumetric flask containing about 80mL of DI add 1mL of stock AccuStandard phosphorus standard. Dilute to 100mL with DI and mix thoroughly. Record the information in the stock standard logbook.

Working Standard Solutions

mL(g) 10mg/L Stock	mg/L P
5.0	0.500
3.0	0.300
1.0	0.100
0.5	0.050
0.25	0.025
0.1	0.010

Transfer aliquots of stock 10mg/L stock as noted above to individual 100mL volumetric flasks. Dilute to volume with DI water and mix thoroughly. Record the information in working standard logbook. Prepare fresh weekly.

Sample Preparation

Sample turbidity is removed by filtration though a 47mm GF/F prior to analysis. Turbidity absorbing in the range of 880 nanometers (nm) will present a positive bias. The analyzed filtrate will yield a total dissolved phosphorus (TDP) result.

The sample is then preserved by freezing at or below -10°C until analysis is performed.

Sample containers are to be rinsed with 1:1 hydrochloric acid, followed by DI water and finally by an aliquot of the sample itself.

Pipette 10mL of sample into a 30mL test tube. 10mL of standards, QC and blanks should also be pipetted. The lowest concentration of standard is pipetted 6 times to allow for the analysis of PQL (Project Quantitative Limit) throughout the run. The rest of the standards are pipetted at least 3 times for each concentration.

The quality control sample (QC) is made up in 100mL volumes per every 60 or so samples. Larger quantities are necessary if running large batches of samples. The QC concentration changes with each new lot # purchased from Environmental Resource Associates (Catalog no. 525). Prepare fresh daily.

Generally, spiked samples are spiked with 250µL of the 10ppm stock standard and are spiked directly into the test tube before digestion yielding a spike concentration of 0.244ppm.

To each test tube add 3mL of digestion reagent and mix thoroughly. Place the samples, QC and standards into the autoclave and heat from 235°F.

Allow the autoclave pressure to equalize, and the temperature to decrease removing the sample. Cool to room temperature overnight.

If analysis cannot be performed immediately samples can be stored at 4°C after digestion.

Transfer the samples to disposable glass test tubes for automated ortho-phosphate analysis on the Lachat.

Instrumental Analysis

Analyze the sample for TDP using Lachat method for phosphate in water and seawater (method 31-115-01-1-H).

It is assumed that the user is basically familiar with the appearance and location of the various parts of the Lachat. It is also assumed that a method for running TDP analyses has already been created, and that the user is familiar with basic system operations. For a physical description of the instrument and any related software information, see the section entitled “The Lachat Quick Chem 8500”.

Ensure that pump tubing is pumping all reagents and proper sample loop is connected.

Ensure that the sample line from the auto-sampler is connected to port 6 of the valve.

Ensure that a method is selected that utilizes the refractive index correction as all TDP samples have the refractive index dip at the start of the peak. This correction eliminates any issues that would be seen with integration.

Calculations

- Percent recovery for the spike is determined using the following formula:

$$\%R = \frac{(A - B)}{C} \times 100$$

Where:
A = measured value in mg/L for the sample + spike
B = measured value in mg/L for the original sample
C = concentration of the spike in mg/L

- Relative percent difference for the duplicate is calculated by the following formula:

$$RPD = \frac{(A - B)}{[(A + B) \div 2]} \times 100$$

Where:
A = the value in mg/L for the first run of the sample
B = the value in mg/L for the second run of the sample

Quality Control

A certified second source quality control sample (purchased from Fisher) is analyzed for every delivery group (or every 10 samples) and the value must be within 85-115% recovery to be considered acceptable.

A spike is analyzed for every delivery group (or every 10 samples). The percent recovery must fall within the 85-115% recovery to be considered acceptable.

A duplicate is analyzed for every delivery group (or every 10 samples) and must have a relative percent difference below 20%.

A blank is analyzed for every delivery group or every 10 samples and the value must fall below the practical quantitation limit (PQL) to be considered acceptable. The concentration of the PQL is the low standard.

A second quality control sample is analyzed at the end of the run and is the concentration of the PQL, or the lowest standard. Acceptable ranges for the PQL are 50-150% recovery.

Other System Notes

Chemistry channel 2
Sample loop 150cm
Light interference filter: 880nm
Special instructions: The reaction module for phosphorus determinations comes equipped with a heating coil that heats the sample stream (after the addition of the reagents) to 37°C, which promotes better color development. This coil should be given 15 minutes to warm up before any samples are run.

Dissolved Silica EPA 370.1

Scope and Application

EPA Method 370.1 is the reference method for measuring dissolved silica in seawater by automated colorimetric determination. This section provides a stepwise procedure for bench use by laboratory personnel.

This method (Lachat silica method number 10-114-27-1-B) was developed for the quantitative analysis of silica in seawater. The applicable range is 0 to 5mg/L. Samples higher than the calibration range must be diluted and re-run or the instrument may be recalibrated with higher concentration standards.

This method is based on automated colorimetric determination and is restricted to the use by or under the supervision of analysts experienced in the use of auto analyzers as a quantitative tool.

Summary of Method

For dissolved silica analysis, the whole water sample is filtered through a polycarbonate 47mm, 0.4µm membrane filter in the field. The filtrate is then refrigerated at 4°C (do not freeze) until analysis can be completed. Analysis is completed within 28 days from arrival date at the laboratory.

Samples for dissolved silica are analyzed by an automated procedure, on Lachat QuickChem 8500 flow analyzer, and is based on the reduction of silicomolybdate in acidic solution to molybdenum blue by ascorbic acid. Oxalic acid is introduced to the sample stream before the addition of ascorbic acid to minimize interference from phosphates and is measured at 820 nm.

The Lachat Auto Analyzer is calibrated with a minimum of a six point curve (including the blank) at the time of analysis (purchased from AccuStandard). The calibration curve is then verified by an external quality control sample from an independent supplier. Supplier guidelines are used for making up the quality control solutions, as well as for information on the true value and acceptable value range for the analytes being measured in each quality control sample.

An initial calibration check along with an initial calibration blank, demonstrate that the instrument is capable of acceptable performance at the beginning of the sample analysis. In order to ensure continuing acceptable performance a continuing calibration check and continuing calibration blank are run every tenth sample. For every 10 samples, a laboratory spike analysis and a laboratory duplicate analysis are performed. At the end of the run a practical quantitative limit (PQL) is run for further quality control verification.

The Method Detection Limit (MDL) and Practical Quantitation Limit (PQL) for this analysis are determined yearly. For current MDL and PQL limits, see the chart entitled Method Detection Limit Determination.

This method is based on automated colorimetric determinations and is restricted to the use by, or under the supervision of, analysts experienced in the use of auto analyzer equipment.

Interferences

Silica contamination is likely to occur if reagents, standards or samples are stored in glass. Keep the use of glass to a minimum, and do not use glass for storage. Use plastic auto analyzer cups on the Lachat.

Phosphate interference is reduced by the addition of oxalic acid.

Tannin and large amounts of sulfides or iron can cause interference. Remove sulfides by acidifying, then boiling the samples. Disodium EDTA will take care of iron interference. This is not typically performed on LISS samples.

References

10-114-27-1-B, October 30, 2007. Determination of Silicate by Flow Injection Analysis. Lachat Instruments, Loveland, Colorado.

EPA Method 370.1. Editorial Revision 1978. Silica, Dissolved (Colorimetric). U.S. Environmental Protection Agency

Associated SOP's

Refer to "SOP" titled "Hazardous Waste SOP" for proper waste disposal.

Refer to the notebook titled "SOP's" located in the nutrients laboratory.

Safety

Reagents are hazardous and are made in the hood. Protective gear is worn when making reagents.

Ensure that waste lines from the Lachat are going to the proper hazardous waste jug located under the instrument.

Refer to the University of Connecticut's Environmental Health and Safety chemical health and safety web page at:

http://www.ehs.uconn.edu/ppp/index.php

A hard copy of the Chemical Hygiene Plan can be found on the notebook rack located on the lab bench. Also refer to the Hazardous Waste SOP.

Materials

Lachat QuickChem 8500 Analyzer

Procedure

Reagent Preparation

Unless otherwise specified, all chemicals should be ACS grade or equivalent. DI water refers to high quality reagent water, TYPE I or TYPE II as defined in ASTM Standards, Part 31, D1193-74. When making reagents, date the container in which the reagent is stored, initial it, and write out the entire name of the reagent.

- Molybdate Reagent

We are currently using Fisher ammonium molybdate (catalog no. A674-500) and Fisher sulfuric acid (catalog no. SA176-4).

Ammonium Molybdate Tetrahydrate	40g
Sulfuric Acid	16mL
DI Water, q.s.	1000mL final vol.

Fill volumetric flask with about 600mL of DI water and add 16mL of sulfuric acid. Cool under tap water, being careful not to introduce the tap water into the volumetric flask. Add 40g ammonium molybdate and dilute to final volume. Degas for a minimum of 5 minutes with helium. If a blue color or precipitate develops, discard the solution. Store the solution in a dark plastic container. The solution is stable for one month.

- Oxalic Acid Reagent

We are currently using Fisher oxalic acid dihydrate (catalog no. A219-500).

Oxalic Acid	50g
DI Water, q.s.	500mL final vol.

Dissolve 50g of oxalic acid in 500mL of DI water and stir to mix, about 30 to 60 minutes. Degas with helium for at least 5 minutes. Store the solution in a clear plastic container. Solution is stable for 1 week.

- Tin Chloride

We are currently using Fisher tin (II) chloride dihydrate (catalog no. T142-100) and hydroxylamine hydrochloride (catalong no. H330-100) and sulfuric acid (catalog no. SA176-4).

Sulfuric Acid	11mL
Hydroxylamine Hydrochloride	1g
Tin (II) Chloride	0.15g
DI Water, q.s.	500mL final vol.

In a 500mL class A volumetric flask, add 250mL DI water, then slowly add 11mL sulfuric acid, 1g hydroxylamine hydrochloride, and tin (II) chloride dilute to volume and mix. Degas for a minimum of 5 minutes. Store the solution in a clear plastic container and is stable for one week.

Standard Preparation

Working standards can be made by weight or volume. Standards are made using a 1000µg/mL stock certified from AccuStandard or another source different from the QC. Prepare fresh weekly.

Stock Standard, 10.0mg/L N

AccuStandard Stock (Silica SiO_2)	2mL
DI water, q.s.	200mL

In a 100mL plastic volumetric flask containing about 80mL of DI add 1mL of stock AccuStandard silica standard. Dilute to 100mL with DI and mix thoroughly. Record the information in the stock standard logbook. Make sure that plastic volumetric flasks are used to prepare all standards and quality controls. There is not a plastic volumetric flask in the 200mL volume, so prepare the stock in duplicate aliquots and mix together.

Working Standard Solutions

mL(g) 10mg/L Stock	mg/L SiO_2
50	5.0
30	3.0
10	1.0
5	0.5
3	0.3
1	0.1
0.5	0.05

Transfer aliquots of stock 10mg/L stock as noted above to individual 100mL volumetric flasks. Dilute to volume with DI water and mix thoroughly. Record the information in working standard logbook. Prepare fresh weekly.

Sample Preparation

Sample turbidity is removed by filtration though a 47mm, 0.4µm polycarbonate membrane filter prior to analysis. Turbidity absorbing in the range of 820 nanometers (nm) will present a positive bias. When filtered sample is analyzed, the dissolved silica result is reported. Dissolved silica values are reported, and the filter is analyzed for biogenic silica.

Preserve the sample by refrigerating at 4°C until the time of analysis.

Ensure that samples are brought to room temperature before analysis.

Generally, 6mL of sample is spiked with 0.015mL of the 1000µg/mL stock standard, yielding a spike concentration of 2.49ppm.

The quality control sample (QC) is made up in 100mL volumes per every 60 or so samples. Larger quantities are necessary if running large batches of samples. Make the QC in a plastic volumetric flask. Generally, the QC concentration is 3.0ppm. Prepare fresh daily.

Sample containers are to be rinsed with 1:1 hydrochloric acid, followed by DI water and finally by an aliquot of the sample itself. Disposable plastic sample tubes are used during analysis to avoid contamination from improper washing of glass tubes.

Instrumental Analysis

It is assumed that the user is basically familiar with the appearance and location of the various parts of the Lachat QuickChem AutoAnalyzer. It is also assumed that a method for running silica analyses has already been created, and that the user is familiar with basic system operations. For a physical description of the instrument and any related software information, see the section entitled "The Lachat Quick Chem 8500".

The silica manifold shares the number 2 channel with ortho-phosphorus and must be installed prior to analysis. It is located in the drawer next to the computer. Ensure that the wavelength filter and heating coil are hooked up to the manifold with the proper sample loop.

Allow the manifold heater to come to temperature before starting analysis.

Check for leaks while pumping rinse water through the manifold. It is common after switching manifolds to have leaking at various fittings. It is crucial to ensure that the leaking has been addressed before moving on to running samples.

Refer to the Lachat SOP for calibrating and setting up the sample run.

Calculations

- Percent recovery for the spike is determined using the following formula:

$$\%\text{R} = \frac{(A - B)}{C} \times 100$$

Where:

A = measured value in mg/L for the sample + spike
B = measured value in mg/L for the original sample
C = concentration of the spike in mg/L

- Relative percent difference for the duplicate is calculated by the following formula:

$$\text{RPD} = \frac{(A - B)}{[(A + B) \div 2]} \times 100$$

Where:

A = the value in mg/L for the first run of the sample
B = the value in mg/L for the second run of the sample

Quality Control

A certified second source quality control sample (purchased from Fisher) is analyzed for every delivery group (or every 10 samples) and the value must be within 85-115% recovery to be considered acceptable.

A spike is analyzed for every delivery group (or every 10 samples). The percent recovery must fall within the 85-115% recovery to be acceptable.

A duplicate sample is analyzed for every delivery group (or every 10 samples). The relative percent difference (RPD) must be below 20%.

A blank is analyzed every delivery group or every 10 samples and the value must fall below the practical quantitation limit (PQL) to be considered acceptable. The concentration of the PQL is the low standard.

A second quality control sample is analyzed at the end of the run and is the concentration of the PQL, or the lowest standard. Acceptable ranges for the PQL are 50-150 % recovery.

Other System Notes

Sample Loop: 150cm
Light interference filter: 820nm
Heater Loop: 175cm

Biogenic Silica EPA 370.1

Scope and Application

The method for the determination of biogenic silica is a polycarbonate membrane filter pad that is digested first with sodium hydroxide and then followed by sulfuric acid neutralization. The digested sample is then analyzed for silica by an automated colorimetric procedure. This section provides a stepwise procedure for bench use by laboratory personnel.

EPA method 370.1 is the reference method for measuring total and dissolved silica in water and seawater by automated colorimetric determination. This section provides a stepwise procedure for bench use by laboratory personnel.

This method (Lachat silica method number 10-114-27-1-B) was developed for the quantitative analysis of silica in water and seawater. The applicable range is 0 to 5mg/L. Samples higher than the calibration range must be diluted and re-run or the instrument may be recalibrated with higher concentration standards.

This method is based on automated colorimetric determination and is restricted to the use by or under the supervision of analysts experienced in the use of auto analyzers as a quantitative tool.

Summary of Method

A known volume of whole water is passed though a 47mm, 0.4µm polycarbonate membrane filter and placed in a plastic centrifuge tube in the field. The filter is frozen until ready for analysis. The digestion of the filter pad is performed within 28 days of arrival at the laboratory. 0.2M sodium hydroxide solution is added to the centrifuge tube with filter. The tube and filter are then heated in a pressure cooker for 15 minutes. The sample is then neutralized with 0.5M sulfuric acid and diluted to a final volume of 50mL with DI water. The sample is then ready for analysis by the silica method on the Lachat.

Samples for biogenic silica are analyzed by an automated procedure, on the Lachat QuickChem 8500 flow analyzer, whereby silica reacts with molybdate reagent in acid media to form a yellow silicomolybdate complex which is measured at 820 nm.

The Lachat Auto Analyzer is calibrated with a minimum of a six-point curve (including the blank) at the time of analysis (purchased from AccuStandard). The calibration curve is then verified by an external quality control sample from an independent supplier. Supplier guidelines are used for making up the quality control solutions, as well as for information on the true value and acceptable value range for the analytes being measured in each quality control sample.

An initial calibration check along with an initial calibration blank, demonstrate that the instrument is capable of acceptable performance at the beginning of the sample analysis. In order to ensure continuing acceptable performance a continuing calibration check and continuing calibration blank are run every tenth sample. For every 10 samples, a laboratory spike analysis and a laboratory duplicate analysis are performed. At the end of the run a PQL (Practical Quantitation Limit) is run for further quality control verification.

The Method Detection Limit (MDL) and Practical Quantitation Limit (PQL) for this analysis are determined yearly. For current MDL and PQL limits, see the chart entitled Method Detection Limit Determination.

This method is based on automated colorimetric determinations and is restricted to the use by, or under the supervision of, analysts experienced in the use of auto analyzer equipment.

Interferences

Silica contamination is likely to occur if reagents, standards or samples are stored in glass. Keep the use of glass to a minimum, and do not use glass for storage.

Phosphate interference is reduced by the addition of oxalic acid.

Tannin and large amounts of sulfides or iron can cause interference. Remove sulfides by acidifying, then boiling the samples. Disodium EDTA will take care of iron interference.

References

10-114-27-1-B, October 30, 2007. Determination of Silicate by Flow Injection Analysis. Lachat Instruments, Loveland, Colorado.

EPA Method 370.1. Editorial Revision 1978. Silica, Dissolved (Colorimetric). U.S. Environmental Protection Agency

Associated SOP's

Refer to "SOP" titled "Hazardous Waste SOP" for proper waste disposal.

Refer to the notebook titled "SOP's" located in the nutrients laboratory.

Safety

Reagents are hazardous and are made in the hood. Protective gear is worn when making reagents.

Ensure that waste lines from the Lachat are going to the proper hazardous waste jug located under the instrument.

Refer to the University of Connecticut's Environmental Health and Safety Chemical Health and Safety web page at:

http://www.ehs.uconn.edu/ppp/index.php

A hard copy of the Chemical Hygiene Plan can be found on the notebook rack located on the lab bench. Also refer to the Hazardous Waste SOP.

Materials

Lachat QuickChem 8500 Auto Analyzer
Pressure cooker
Hot Plate

Procedure

Reagent Preparation

Unless otherwise specified, all chemicals should be ACS grade or equivalent. DI water refers to high quality reagent water, TYPE I or TYPE II as defined in ASTM Standards, Part 31, D1193-74. When making reagents, date the container in which the reagent is stored, initial it, and write out the entire name of the reagent.

- Molybdate Reagent

We are currently using Fisher ammonium molybdate (catalog no. A674-500) and Fisher sulfuric acid (catalog no. SA176-4).

Ammonium Molybdate Tetrahydrate	40g
Sulfuric Acid	16mL
DI Water, q.s.	1000mL final vol.

Fill volumetric flask with about 600mL of DI water and add 16mL of sulfuric acid. Cool under tap water, being careful not to introduce the tap water into the volumetric flask. Add 40g ammonium molybdate and dilute to final volume. Degas for a minimum of 5 minutes with helium. If a blue color or precipitate develops, discard the solution. Store the solution in a dark plastic container. The solution is stable for one month.

- Oxalic Acid Reagent

We are currently using Fisher oxalic acid dihydrate (catalog no. A219-500).

Oxalic Acid	50g
DI Water, q.s.	500mL final vol.

Dissolve 50g of oxalic acid in 1000mL of DI water and stir to mix, about 30 to 60 minutes. Degas with helium for at least 5 minutes. Store the solution in a clear plastic container. Solution is stable for 1 week.

- Tin Chloride

We are currently using Fisher tin (II) chloride dihydrate (catalog no. T142-100) and hydroxylamine hydrochloride (catalog no. H330-100) and sulfuric acid (catalog no. SA176-4).

Sulfuric Acid	11mL
Hydroxylamine Hydrochloride	1g
Tin (II) Chloride	0.15g
DI Water, q.s.	500mL final vol.

In a 1L class A volumetric flask, add 900mL DI water, then slowly add 50g L-ascorbic acid, dilute to volume and mix. The solution is stable for one week.

- 1N Sulfuric Acid

We are currently using Fisher sulfuric acid (catalog no. SA176-4),

Sulfuric Acid	28mL
DI Water, q.s.	1000mL final vol.

In a 500mL class A volumetric flask, add 250mL DI water, then slowly add 11mL sulfuric acid, 1g hydroxylamine hydrochloride, and tin (II) chloride dilute to volume and mix. Degas for a minimum of 5 minutes. Store the solution in a clear plastic container. Solution is stable indefinitely.

Digestion Reagents:

- 0.2M Sodium Hydroxide

Sodium Hydroxide	8g
DI Water, q.s.	1000mL final vol.

Fill a 1 L clear small mouth plastic poly bottle with 1000mL of DI water and add 8g of sodium hydroxide. Prepare prior to use.

- 0.5M Sulfuric Acid

Sulfuric Acid	14mL
DI Water, q.s.	1000mL final vol.

Fill a 1L clear small mouth plastic poly bottle with 1000mL of DI water and add 14mL of sulfuric acid. Prepare prior to use.

Standard Preparation

Working standards can be made by weight or volume. Standards are made using a 1000µg/mL stock certified from AccuStandard or another source different from the QC. Prepare fresh weekly.

Stock Standard, 10.0mg/L N

AccuStandard Stock (Silica)	2mL
DI Water, q.s.	200mL

In a 100mL plastic volumetric flask containing about 80mL of DI add 1mL of stock AccuStandard silica standard. Dilute to 100mL with DI and mix thoroughly. Record the information in the stock standard logbook. Make sure that plastic volumetric flasks are used to prepare all standards and quality controls. There is not a plastic volumetric flask in the 200mL volume, so prepare the stock in duplicate aliquots and mix together.

Working Standard Solutions

mL(g) 10mg/L Stock	mg/L SiO_2
50	5.0
30	3.0
10	1.0
5	0.5
3	0.3
1	0.1
0.5	0.05

Transfer aliquots of stock 10mg/L stock as noted above to individual 100mL volumetric flasks. Dilute to volume with DI water and mix thoroughly. Record the information in working standard logbook. Prepare fresh weekly.

Sample Preparation

Preserve the sample by freezing at or below -10°C until the time of analysis.

Clean, sterilized blue capped preparation tubes are purchased from Fisher Scientific (cat #010-500-263) and provided to CT DEEP to collect the filter. One filter is placed in the tube and stored in the freezer prior to analysis.

Ensure that samples are brought to room temperature before analysis.

Generally, 6mL of sample is spiked with 0.015mL of the 1000µg/mL stock standard, yielding a spike concentration of 2.49ppm.

The quality control sample (QC) is made up in 100mL volumes per every 60 or so samples. Larger quantities are necessary if running large batches of samples. Make the QC in a plastic volumetric flask. Generally, the QC concentration is 3.0ppm. Prepare fresh daily.

Sample Digestion

Line up blue capped, sample tubes in sample rack with corners cut off (green) so they will fit in the pressure cooker.

Set pressure cooker on hot plate, set on high with about 1-2 inches of tap water in the bottom. Wait until water is boiling before adding samples.

Add 8mL of the first digestion reagent (0.2M sodium hydroxide) and make sure that the filter is submerged.

Place one tray at a time in pressure cooker for 15 minutes.

Add 40mL of DI water to boiled samples using a plastic small mouth bottle and dispensette pipette.

Add 2mL of 0.5M sulfuric acid

Invert three times and let samples sit overnight before analyzing the next day.

ALWAYS USE PLASTIC FOR EVERYTHING (IE. SAMPLE CUPS, QC VOLUMETRIC FLASK AND REAGENTS)!!

Instrumental Analysis

It is assumed that the user is basically familiar with the appearance and location of the various parts of the Lachat QuickChem AutoAnalyzer. It is also assumed that a method for running silica analyses has already been created, and that the user is familiar with basic system operations. For a physical description of the instrument and any related software information, see the section entitled “The Lachat Quick Chem 8500”.

The silica manifold shares the number 2 channel with ortho-phosphorus and must be installed prior to analysis. It is located in the drawer under the hood. Ensure that the wavelength filter and heating coil are hooked up to the manifold with the proper sample loop.

Allow the manifold heater to come to temperature before starting analysis.

Check for leaks while pumping rinse water through the manifold. It is common after switching manifolds to have leaking at various fittings. It is crucial to ensure that the leaking has been addressed before moving on to running samples.

The same QC is used for both the biogenic silica and the dissolved silica.

The same calibration is used when running both dissolved and biogenic silica and the samples may be run at the same time.

Refer to the Lachat SOP for calibrating and setting up the sample run.

Calculations

- Percent recovery for the spike is determined using the following formula:

$$\%R = \frac{(A - B)}{C} \times 100$$

Where:

A = measured value in mg/L for the sample + spike
B = measured value in mg/L for the original sample
C = concentration of the spike in mg/L

- Relative percent difference for the duplicate is calculated by the following formula:

$$RPD = \frac{(A - B)}{[(A + B) \div 2]} \times 100$$

Where:

A = the value in mg/L for the first run of the sample
B = the value in mg/L for the second run of the sample

- Biogenic Silica is calculated by the following formula:

$$\text{BioSiO2 (mg/L)} = \frac{(A \times B)}{\text{Volume Filtered } (L)}$$

Where:

A = instrument reading (mg/L)
B = volume extracted (L)

Quality Control

A certified second source quality control sample (purchased from Fisher) is analyzed for every delivery group (or every 10 samples) and the value must be within 85-115% recovery to be considered acceptable.

A spike is analyzed for every delivery group (or every 10 samples). The percent recovery must fall within the 85-115% recovery to be acceptable.

A duplicate sample is analyzed for every delivery group (or every 10 samples). The relative percent difference (RPD) must be below 20%.

A blank is analyzed every delivery group or every 10 samples and the value must fall below the practical quantitation limit (PQL) to be considered acceptable. The concentration of the PQL is the low standard.

A second quality control sample is analyzed at the end of the run and is the concentration of the PQL, or the lowest standard. Acceptable ranges for the PQL are 50-150% recovery.

Other System Notes

Sample Loop: 150cm
Light interference filter: 820nm
Heater Loop: 175cm

Particulate Phosphorus EPA 365.1

Scope and Application

Aspila et al., 1976, is the reference method for acid extraction of particulate phosphorus deposited on filters. Ortho-phosphorus is the sole P product of the extraction and is determined by an automated colorimetric procedure. This section provides a stepwise procedure for bench use by laboratory personnel.

The Method Detection Limit (MDL) and Practical Quantitation Limit (PQL) for this analysis are determined yearly. For current MDL and PQL limits, see the chart entitled Method Detection Limit Determination.

A known volume of whole water is passed through two 47mm GF/F filters in the field. The filters are frozen until ready for analysis within 28 days. When ready, they are dried in a drying oven for one hour at 105°C and muffled at 500°C in a muffle furnace for one hour. The filters are placed in a screw cap test tube and extracted with 30mL of 1N hydrochloric acid, capped and inverted repeatedly. The sample is filtered before analysis with a syringe and 0.45μm sterile filter into auto analyzer cups. The sample is then ready for analysis by the ortho-phosphate method on the Lachat.

For every 10 samples, a blank, a laboratory spike analysis and a laboratory duplicate analysis are performed.

The analysis depends on the formation of a phosphomolybdenum blue complex, which is read colorimetrically at 880nm.

Phosphomolybdate Complex: $12MoO_3 + H_2PO_4^- \rightarrow (H_2PMo_{12}O_{40})^-$

This method is based on automated colorimetric determinations and is restricted to the use by, or under the supervision of, analysts experienced in the use of auto analyzer equipment.

The Lachat Analyzer is calibrated with a minimum of a six-point curve (including the blank) at the time of analysis (purchased from AccuStandard). The calibration curve is then verified by an external quality control sample purchased from Fisher (Ricca Cat # 5839.1-16). Supplier guidelines are used for making up the quality control solutions, as well as for information on the true value and acceptable value range for the analytes being measured in each quality control sample.

An initial calibration check along with an initial calibration blank, demonstrate that the instrument is capable of acceptable performance at the beginning of the sample analysis. In order to ensure continuing acceptable performance a continuing calibration check and continuing calibration blank are run every tenth sample. For every 10 samples, a laboratory spike analysis and a laboratory duplicate analysis are performed. At the end of the run a PQL (Practical Quantitation Limit) is run for further quality control verification.

Interferences

Arsenate is analyzed similarly to ortho-phosphate and will cause interference if present. Reducing the arsenic acid to arsenious acid with sodium bisulfite should alleviate this

problem. Sodium bisulfite treatment will also take care of any problems with high iron concentration (>50mg/L).
Any silica present will react with the reagents in this method, forming a pale blue complex which also absorbs at 880nm. Because the method is very sensitive to small amounts of phosphorus, sensitivity to silica is also high. A silica concentration of approximately 30mg/L would be required to produce a 0.005mgP/L positive error of ortho-phosphate. Samples containing higher than this concentration of silica would need to be addressed.

Glass should be avoided if possible. Reagents should be made and stored in plastic. Naturally occurring silica in samples may be discriminated against by using an alternate color reagent recipe, given below, although this is not standard procedure.

Acidity among samples, standards and blanks should be carefully controlled. Large variations in acidity will affect sample and/or standard peaks.

Good glassware cleaning procedures should always be used. Phosphorus contamination is a constant problem. Proper glassware washing protocol should elevate this problem.

References

31-115-01-1-H, August 2003. Determination of Orthophosphate by Flow Injection Analysis. Lachat Instruments, Loveland, CO.
EPA Method 365.1. Determination of Phosphorus by Semi-Automated Colorimetry. Revision 2.0, August 1993. Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Standard Method 4500-P A, B, G and H Phosphorous: Flow Injection Method. Page 4-139 – 4-153, 20th Edition, 1998, Standard Methods for the Examination of Water and Wastewater.

Associated SOP's

Refer to "SOP" titled "Hazardous Waste SOP" for proper waste disposal.

Refer to the notebook titled "SOP's" located in the nutrients laboratory.

Safety

Samples are disposed in hazardous waste jugs that are properly labeled.

Reagents are hazardous and are made in the hood. Protective gear is worn when making reagents.

Ensure that waste lines from the Lachat are going to the proper hazardous waste jug located under the instrument.

Refer to the University of Connecticut's Environmental Health and Safety Chemical Health and Safety web page at:

http://www.ehs.uconn.edu/ppp/index.php

A hard copy of the Chemical Hygiene Plan can be found on the notebook rack located on the lab bench. Also refer to the Hazardous Waste SOP.

Materials

Drying Oven (Fisher model 750G)
Muffle Furnace (Lindberg type 51828)
Lachat QuickChem 8500 Analyzer

Procedure

Reagent Preparation

Unless otherwise specified, all chemicals should be ACS grade or equivalent. DI water refers to high quality reagent water, TYPE I or TYPE II as defined in ASTM Standards, Part 31, D1193-74. When making reagents, date the container in which the reagent is stored, initial it, and write out the entire name of the reagent.

- Stock Antimony Potassium Tartrate

We are currently using Fisher antimony potassium tartrate (catalog no. A867-250).

Antimony Potassium Tartrate	1.61g
DI Water, q.s.	500mL final volume

Dissolve 1.61 g of antimony potassium tartrate in about 400mL of DI water in 500mL volumetric flask. Dilute to 100mL with DI water and mix thoroughly. Store the solution in a dark plastic container. Solution is stable for one month.

- Stock Ammonium Molybdate

We are currently using Fisher ammonium molybdate (catalog no. A674-500).

Ammonium Molybdate	20g
DI Water, q.s.	500mL final volume

Fill amber poly bottle ¾ with DI water and add 20g of ammonium molybdate. Dilute to 500mL with DI water and mix thoroughly. Solution is stable for one month.

- Molybdate Color Reagent—PP version.

This reagent differs slightly from the other ortho-phosphate reagent recipe. Because the samples are acidic, omit the sulfuric acid from this reagent.

Stock Antimony Potassium Tartrate Solution	72mL
Stock Ammonium Molybdate	213mL
DI Water, q.s.	1000mL final volume

To a 1 liter volumetric flask add about 500mL of DI water, then add 213mL of stock ammonium molybdate solution and 72mL of stock antimony potassium tartrate solution. Dilute to 1000mL with DI water and mix thoroughly. Degas with helium for at least 5 minutes. Store in a dark plastic container. This solution is stable for one month.

- Ascorbic Acid

We are currently using Fisher, L-ascorbic acid (catalog no. BP351-500) and Fisher SDS (catalog no. BP166-100).

Ascorbic Acid	60g
SDS	1g
DI Water, q.s.	1000mL final volume

In a 1L volumetric add 60g of ascorbic acid in approximately 800mL DI water. Dilute to 1000 mL with DI water and mix thoroughly. Degas for a minimum of 5 minutes. Pour into clear plastic bottle and add 1g of SDS and swirl gently. This solution is stable for 5 days. Store the solution in a clear poly container.

- 1N Hydrochloric Acid

We are currently using Fisher hydrochloric acid (catalog no.A144S-212). The HCl should be made in a large enough batches to cover the extraction and the making of the standards, blanks and QC. 2 liters is a good amount for a typical sample batch. The HCl can be made in an extra large beaker and transferred to smaller bottles.

Hydrochloric Acid	172mL
DI Water	2000mL final volume

Fill ¾ with DI water and cautiously, while stirring, slowly add 172mL of hydrochloric acid. Bring to final volume of 2L. Cool to room temperature and transfer to smaller bottles. Prepare fresh quarterly.

Standard Preparation

Working standards can be made by weight or volume. Standards are made using a 1000µg/mL stock certified from AccuStandard or another source different from the QC and are made from 1N HCL. Prepare fresh weekly.

Stock Standard, 10.0mg/L N

AccuStandard Stock (Phosphorous)	1mL
1N HCl	100mL final volume

In a 100mL volumetric flask containing about 80mL of 1N HCl add 1mL of stock AccuStandard phosphorus standard. Dilute to 100mL with 1N HCL and mix thoroughly. Record the information in the stock standard logbook.

Working Standard Solutions

mL(g) 10mg/L Stock	mg/L P
10	1.0
5	0.5
3	0.3
1	0.1
0.5	0.05
0.25	0.025

Transfer aliquots of stock 10mg/L stock as noted above to individual 100mL volumetric flasks. Dilute to volume with 1N HCl and mix thoroughly. Record the information in working standard logbook. Prepare fresh weekly.

Sample Preparation

Sample Digestion

If not completed in the field, filter in duplicate 500mL of whole water sample through a 47mm GF/F filter (Fisher Cat # 09-874-71). Keep the filters frozen at or below -10°C until analysis.

Generally, spiked samples are spiked with 100µL of the 10ppm stock standard, yielding a spike concentration of 0.196ppm.

The quality control sample (QC) is made up in 100mL volumes per every 60 or so samples. Larger quantities are necessary if running large batches of samples. Generally, the concentration of the QC is 0.3ppm and is made with 1N HCL. Prepare fresh daily.

Dry the filters for a minimum of one hour at 105°C, although overnight is preferable, and keep in desiccator until analysis.

Muffle the filter at 500°C for 1 hour by placing in a crucible and recording proper crucible ID numbers in the preparation logbook.

After cooling, place both filters from each site into the same 50mL digestion tube with 30mL of 1N HCl.

Filter the sample with disposable syringe and 0.45µm sterile nylon filter directly into the Lachat auto analyzer cups.

Instrumental Analysis

Analyze the sample for ortho-phosphosphorus using the Lachat ortho-phosphate method. It is assumed that the user is basically familiar with the appearance and location of the various parts of the Lachat. It is also assumed that a method for running particulate phosphorus analyses has already been created, and that the user is familiar with basic system operations. For more information on the creation of Lachat methods and on basic operations see the section entitled “The Lachat Quick Chem 8500”.

Ensure that pump tubing is pumping all reagents and proper sample loop is connected.

Ensure that the sample line from the auto-sampler is connected to port 6 of the valve.

Calculations

- Percent recovery for the spike is determined using the following formula:

$$\%R = \frac{(A - B)}{C} \times 100$$

Where:

A = measured value in mg/L for the sample + spike
B = measured value in mg/L for the original sample
C = concentration of the spike in mg/

- Relative percent difference for the duplicate is calculated by the following formula:

$$\text{RPD} = \frac{(A - B)}{[(A + B) \div 2]} \times 100$$

Where:
A = the value in mg/L for the first run of the sample
B = the value in mg/L for the second run of the sample

- Particulate Phosphorus is calculated by the following formula:

$$\text{PP (mg/L)} = \frac{(\text{A} \times \text{B})}{\text{Volume Filtered (L)}}$$

Where:
A = instrument reading (mg/L)
B = volume extracted (L)

Quality Control

A certified second source quality control sample (purchased from Fisher) is analyzed for every delivery group (or every 10 samples) and the value must be within 85-115% recovery to be considered acceptable. The QC is made with 1N hydrochloric acid.

A spike is analyzed for every delivery group (or every 10 samples). The percent recovery must fall within the 85-115% recovery to be considered acceptable.

A duplicate is analyzed for every delivery group (or every 10 samples) and the relative percent difference must fall below 20%.

A blank is analyzed every delivery group, or every 10 samples and the value must fall below the PQL to be acceptable. The blank is made with 1N hydrochloric acid.

A second quality control sample is analyzed at the end of the run and is the concentration of the PQL, or the lowest standard. Acceptable ranges for the PQL are 50-150% recovery.

Other System Notes

Chemistry Module 1-1
Sample loop: 150cm
Light interference filter: 880nm
Special instructions: The reaction module for phosphorus determinations comes equipped with a heating coil that heats the sample stream (after the addition of the reagents) to 39°C, which promotes better color development. This coil should be given 15 minutes to warm up before any samples are run.

Total Suspended Solids EPA 160.2

Scope and Application

The method or total suspended solids is also referred to as "Non-Filterable Residue" and is defined as those solids that are retained by a glass fiber filter and dried to constant weight at 103° to 105°C.

The Method Detection Limit (MDL) and Practical Quantitation Limit (PQL) for this analysis are determined yearly. For current MDL and PQL limits, see the chart entitled Method Detection Limit Determination.

References

Standard Method 2540D. Total Suspended Solids Dried at 103-105°C. Page 2-56—2-58, 20th Edition, 1998, Standard Methods for the Examination of Water and Wastewater.

Method 160.2. Total Suspended Solids Dried at 103-105°C. Environmental Protection Agency.

Associated SOP's

Refer to "SOP" titled "Hazardous Waste SOP" for proper waste disposal.

Refer to the notebook titled "SOP's" located in the nutrients laboratory.

Safety and Waste Disposal

Refer to the University of Connecticut's Environmental Health and Safety Chemical Health and Safety web page at:

http://www.ehs.uconn.edu/ppp/index.php

A hard copy of the Chemical Hygiene Plan can be found on the notebook rack located on the lab bench. Also refer to the Hazardous Waste SOP.

Interferences

Exclude large particles or submerged agglomerates of non-homogeneous materials from the sample if it is determined that their inclusion is not desired in the final result.

For samples high in dissolved solids, thoroughly wash the filter to ensure removal of the dissolved material. Prolonged filtering times resulting from filter clogging may produce higher results owing to increased colloidal materials captured on the clogged filter.

Too much residue on the filter may cause a water-entrapping crust; limit the sample size to that yielding no more than 200mg residue.

Materials

Whatman ProWeigh GF/F glass fiber filters (Fisher Cat # 09-735-56)
Mettler Analytical Balance
Drying Oven
Desiccator

Sample Handling

Analysis of whole water samples should begin as soon as possible not to exceed 7 days. Refrigeration or icing to 4°C is recommended for unfiltered samples.

Filters for LISS are delivered and stored in the freezer at or below -10°C until time of analysis.

Procedure

Samples are immediately prepped for TSS analysis. Refrigerate sample at 4°C up to the time of analysis to minimize microbiological decomposition of solids. In no case hold sample for more than 7 days before filtering.

The filtering apparatus is rinsed with 1N hydrochloric acid and then rinsed three times with DI Water using a beaker to catch filtered water for wastes. Place a TSS pre-weighed filter on filtering platform, and rinse with DI Water. Record initial filter weight and tin ID in the logbook. Write the sample ID on the tin with a sharpie.

Shake sample bottle well.

Pour 500mL of sample into a clean, acid washed graduated cylinder and pour into the filtering apparatus.

Limit the sample size to that yielding no more than 200mg of residue because excessive residue on the filter may form a water-entrapping crust.

Pour sample into the filtering apparatus and rinse graduated cylinder 3 times with at least 50mL of DI water and pour into the filtering cup. Then, rinse the sides of the filtering cup with DI Water to ensure all particulate matter is captured on the TSS filter. Leave the filter on the filtering apparatus until dry before taking it off.

Fold the filter in half so as not to lose any of the particulates. Place filter in tin and cook in oven at 103°-105°C overnight (approximately 16 hours), and then place in desiccator until cool before weighing. Record date, analyst, tin ID, initial weight, and volume filtered in the logbook.

Calibrate balance by pressing and holding the zero button until the display flashes “100g”. Move the sliding knob on the right side of the balance all of the way to the back. Wait until the balance flashes “0”, then slide the knob forward and wait. When finished, the balance should read 0.0000g. Use calibrated weights to verify the calibration of the balance and to check for accuracy. Record the values in the balance verification logbook. Weigh filters and record final weights in logbook.

Ensure that a constant weight has been reached by putting the filters back in the oven for 1 hour and then into the desiccator. After the 2 hour cool down period, record the second weight. When new projects or unknown samples are analyzed, they must be baked,

weighed, and then re-baked and re-weighed until a constant weight is achieved, change being either less that 4% of previous weight, or 0.5mg, whichever is less.

For LISS and CT DEEP river samples, a comparative study has been performed in which no significant difference in weights was observed between initial sample weights and either wt. #1 (samples baked overnight) or wt. #2 (samples re-baked for 1 hour after overnight bake). Therefore, after an overnight baking, samples for these projects do not need to be re-baked and re-weighed. This data is available upon request.

Calculations

- To calculate TSS:

$$\text{mg TSS/L} = \frac{(A - B) \times 1000000}{\text{sample volume (mL)}}$$

Where:

A = weight of filter and residue in grams
B = weight of original filter in grams

- Relative percent difference for the duplicate is calculated by the following formula:

$$\text{RPD} = \frac{(A - B)}{[(A + B) \div 2]} \times 100$$

Where:

A = value in mg/L for the first run of the sample
B = value in mg/L for the second run of the sample

Quality Control

A certified quality control sample is purchased from ERA (CAT# 510 Small Lab Minerals) and is filtered along with samples. It is run every delivery group, or every 20 samples and the value must be within 85-115% recovery to be considered acceptable.

A duplicate is filtered per delivery group or every 20 samples and must have an RPD below 20% to be considered acceptable.

A blank is filtered per delivery group, or every 20 samples and the value must fall below the PQL to be considered acceptable.

Chlorophyll EPA 445.0

Scope and Application

EPA Method 445.0 is the reference method for measuring chlorophyll-a in seawater by flourometric analysis. This section provides a stepwise procedure for bench use by laboratory personnel.

This method for determining chlorophyll-a is more sensitive than the spectrophotometric method.

The fluorometer is calibrated with purchased chlorophyll-a standards of known value from Turner Designs (Cat. # 10-850).

The Method Detection Limit (MDL) and Practical Quantitation Limit (PQL) for this analysis are determined yearly. For current MDL and PQL limits, see the chart entitled Method Detection Limit Determination.

A Chla-NA module is used in the instrument which eliminates the need for the acidification step in the EPA method.

Summary of Method

A known volume of water is filtered through a 25mm, 0.7µm GF/F filter and the resulting pigments are extracted with a 90% acetone solution. The fluorescence of the extract is determined with a fluorometer and the chlorophyll-a concentration is calculated.

Fluorescence is temperature dependent with higher sensitivity occurring at lower temperatures. Samples, standards, blanks and spikes must all be at the same temperature.

Interferences

Any substance extracted for the filter or acquired from laboratory contamination that fluoresces in the red region of the spectrum may interfere in the accurate measurement of chlorophyll-a.

References

EPA Method 445.0. *In Vitro* Determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Algae by Fluorescence. Revision 1.2, September, 1997. National Exposure Research Laboratory, Office of R &D, U.S.E.P.A., Cincinnati, OH 45268

Associated SOP's

Refer to "SOP" titled "Hazardous Waste SOP" for proper waste disposal.

Refer to the notebook titled "SOP's" located in the nutrients laboratory.

Safety

Acetone is extremely flammable and is an eye, mucous and skin irritant. Wear appropriate safety glasses and gloves and use in the hood.

Refer to the University of Connecticut's Environmental Health and Safety Chemical Health and Safety web page at:

http://www.ehs.uconn.edu/ppp/index.php

A hard copy of the Chemical Hygiene Plan can be found on the notebook rack located on the lab bench. Also refer to the Hazardous Waste SOP.

Materials

Turner Trilogy Fluorometer

Procedure

Reagent Preparation

Unless otherwise specified, all chemicals should be ACS grade or equivalent. Deionized water refers to high quality reagent water, Type I or Type II as defined in ASTM Standards, Part 31, and section D1193-75.

- Aqueous Acetone

Acetone	90mL
Deionized Water	10mL

Combine 90mL of acetone with 10mL of deionized water and mix thoroughly. We are currently using Fisher acetone (catalog no. A949-4). Solution is stable for 3 months.

Standard Preparation

We are currently using primary chlorophyll-a standards purchased from Turner Designs (Cat. # 10-850). The two concentrations come in a high and low concentration, and the analyst must dilute the high standard to create a mid-range calibration point.

It is important to pay close attention to pouring chlorophyll standards, to be careful not to lose sample volume. Acetone rapidly evaporates. Be sure to keep all vials capped tightly at all times.

Sample Preparation

A known volume of water is passed through a 25mm, 0.7µm GF/F filter pad in the field. The pad is then folded in half and stored in aluminum foil, labeled, and frozen at or below -10°C until time of analysis. Filters can be stored frozen for 28 days without sample degradation.

Samples may also be filtered in the laboratory and appropriate volumes of sample filtered are recorded and apparatus is rinsed with copious amounts of deionized water before each sample is filtered. Unfiltered water samples are stored in brown bottles in the dark at 4ºC. and should be filtered within 24 hours.

Filter Extraction

Before analysis, the filter pad is thawed and then placed in a 30mL glass screw top test tube.

Next, add 10mL of 90% aqueous acetone, ensure filter is submerged and allow sample to steep.

The tube is then gently shaken and allowed to steep for 1 hour before gently shaken again. The samples are allowed to extract for not more than 24 hours in the dark, in the freezer.

Avoid any direct light throughout this analysis as it can increase the chlorophyll concentration!!!

Instrumental Analysis

It is assumed that the user is basically familiar with the appearance and location of the various parts of the Turner 450 fluorometer.

The tubes are removed from the freezer with the standards and spiking solution and are allowed to warm to room temperature under dark conditions (typically in the cabinet under the hood) until warm so no condensation appears on the outside of the glass test tube.

Calibration of the Fluorometer

Primary chlorophyll standards are purchased from Turner Designs, 845 W. Maude Avenue, Sunnyvale, CA 94085, and are supplied in 20mL vials (Cat # 10-850).

Prepare dilutions of the extracts using 90% acetone to provide concentrations in the appropriate range of µg/L of chlorophyll-a.

It is important not to leave test tube caps open for any length of time as acetone evaporates quickly and this will alter the chlorophyll-a reading.

- On the Trilogy fluorometer select Calibrate → New → ug/L
- Use a test tube with 90% acetone for the blank, make sure to wipe down the test tube with a Kimwipe before placing it to be read.
- Next pour the standards, lowest to highest in three separate test tubes and read.
    - Enter standard concentration → OK → Enter more Stds
    - Continue with calibration? → yes, save calibration as date (mmddyy)

Sample Analysis

The adjustable solid secondary standard (QC2NDCOURC) provides a very stable fluorescent signal. It has an adjustment screw so that it can be tuned to match sample concentrations. It is used to check the stability of the instrument as a second source quality control sample. Control charts are kept to verify a valid acceptance range over time. A chart in the beginning of the CHLa logbook is updated periodically with new acceptance criterion.

Then a low standard (CCV) and blank are used every 20 samples as a continuing calibration verification.

- Press sample ID, the first sample is the QC, the second is the Blank, then the actual samples. Type QC2NDSOURC for the sample name. The 2nd source QC is located

in the small black box next to the machine. The black square test tube holder in the instrument needs to be removed before inserting the QC.

- Press “Measure fluorescence”. For the QC and Blank the volume filtered = 1mL, the solvent added = 1mL. For all other samples the volume filtered = check the PREP LOGBOOK and the solvent added = 10mL.

- After you have finished reading all the samples (including a duplicate per 20 samples, one LCS, and one SPK per every 40 samples) save the Excel worksheet on a removable flash drive as Chla+date (mmddyy).

- For the LCS and SPK only use LISS samples:
    - LCS = 2mL of spike solution in 3mL of 90% acetone
    - SPK = 2mL of spike solution in 3mL of sample (record sample number)
    - When measuring the LCS and SPK the volume filtered (for both) is the same as the volume filtered for the sample that is being used for the SPK.
    - The volume of the solvent is 10mL

- For LISS Samples:
    - Filtered sample volume = 400mL
    - Solvent volume = 10mL
    - This is because there are two filters that each have 200mL filter volume.

Calculations

- Percent recovery for the spike is determined using the following formula:

$$\%R = \frac{(A - B)}{C} \times 100$$

Where:

A = measured value in µg/L for the sample + spike
B = measured value in µg/L for the original sample
C = concentration of the spike in µg/L

- Relative percent difference for the duplicate is calculated by the following formula:

$$RPD = \frac{(A - B)}{[(A + B) \div 2]} \times 100$$

Where:

A = value in µg/L for the first run of the sample
B = value in µg/L for the second run of the sample

Quality Control

A laboratory control sample (LCS) is run for every batch of samples. The spiking solution is made by combining a few high concentration samples and mixing thoroughly. This is then spiked to 90% acetone as the LCS to verify the concentration of the spike. Typically, 2mL of the spiking solution is added to 3mL of either the 90% acetone for the LCS sample or 3mL of sample for the spiked sample.

One of the standards is run throughout the sample analysis as a quality control sample and is analyzed for every delivery group (or every 20 samples) and the value must be within 85-115% recovery to be considered acceptable.

A duplicate is analyzed for every delivery group (or every 40 samples). The duplicate relative percent difference (RPD) must be below 20%.

A blank is analyzed every delivery group or every 20 samples and the value must fall below the PQL to be considered acceptable.

A solid secondary standard cell is run with every batch. Control charts are updated periodically and acceptable ranges are found in the front of the CHLa logbook.

Dissolved Organic Carbon EPA 415.1

Scope and Application

The organic carbon in water is composed of a variety of organic compounds in various oxidation states. To determine the quantity of organically bound carbon the organic molecules must be broken down to a single carbon unit and converted to a single molecular form that can be measured quantitatively.

In this method, total carbon (TC) and total inorganic carbon (TIC) are converted to carbon dioxide (CO_2) at 680°C using a platinum catalyst bed in the reaction chamber. Detection is by non-dispersive infrared (NDIR). The holding time is 28 days.

Summary of Method

Non-Purgeable Organic Carbon (NPOC) values are obtained by acidifying the sample with 1 mol/L hydrochloric acid to a pH of 2 to 3. Sparge gas (Ultra Zero Compressed Air) is bubbled through the sample to eliminate the IC component and the remaining TC is measured in the method described above. This analysis value is referred to NPOC to distinguish it from the TOC value obtained by calculating the difference between TC and IC. It is the non-purgeable organic carbon that is present in a sample in non-volatile form. In the Nutrients Laboratory, NPOC is referred to as TOC as reporting calculated TOC values have not been requested by clients.

Dissolved organic carbon (DOC) is measured by filtering the sample with a 47mm GF/F filter and purging off TIC. LISS samples are run in this manner.

The Method Detection Limit (MDL) and Practical Quantitation Limit (PQL) for this analysis are determined yearly. For current MDL and PQL limits, see the chart entitled Method Detection Limit Determination.

Definitions

NDIR (Non-Dispersive Infrared) - A polyatomic molecule such as CO_2 absorbs infrared radiation of different wavelengths depending upon the bonding condition or kind of atoms comprising the molecule. The number of rays absorbed is directly proportional to the density of the gas according to Lambert-Beer's Law. The density of the gas can be obtained by measuring the amount of rays absorbed by using a dual beam photometer that employs the principle of pressure difference between two detector chambers resulting in an analog signal.

Purge - Acidification of a water sample, which converts inorganic carbon to carbon dioxide gas.

References

TOC-L Total Organic Carbon Analyzer Manual. Shimadzu 2010.

Method 415.1. Organic Carbon, Total (Combustion or Oxidation). Editorial Revision 1974. Environmental Protection Agency.

Standard Method 5310 A & B. Total Organic Carbon by High-Temperature Combustion. Page 5-18—5-22, 20th Edition, 1998, Standard Methods for the Examination of Water and Wastewater.

Associated SOP's

Refer to "SOP" titled "Hazardous Waste SOP" for proper waste disposal.

Refer to the notebook titled "SOP's" located in the nutrients laboratory.

Safety

Refer to the University of Connecticut's Environmental Health and Safety Chemical Health and Safety web page at:

http://www.ehs.uconn.edu/ppp/index.php

A hard copy of the Chemical Hygiene Plan can be found on the notebook rack located on the lab bench. Also refer to the Hazardous Waste SOP.

When packing tubes, use proper protective gear and work in hood as reagents are hazardous.

The waste of the instrument is captured in a hazardous waste bin and labeled "pH < 1".

Materials

Shimadzu Total Organic Carbon Analyzer - Model TOC-L
ASI-L Shimadzu Autosampler

Procedure

Standard Preparation

Deionized water (DI) refers to high quality reagent water, TYPE I or TYPE II as defined in ASTM Standards. Use DI water to prepare all standards. They are made fresh daily.

Dissolved Organic Carbon Standards

Transfer aliquots of AccuStandard 1000µg/mL stock as noted below to individual 100mL volumetric flasks. This standard is certified and from AccuStandard and is kept in the standards drawer. Dilute to final volume of 100mL with DI water and mix thoroughly. Record in standard logbooks. Prepare fresh daily.

Final Standard Conc. (ppm)	Volume of Stock (mg/L)
1.0	0.1
5.0	0.5
10.0	1.0

Reagents and gas

- Hydrochloric Acid—Used for pre-acidification (1mol/L)

Fill 250mL volumetric flask roughly half-way with DI water. Add 20.5mL of concentrated HCl and dilute to final volume.

- 2M Hydrochloric Acid—used to recondition the catalyst

Add 168mL of HCl to a 1 Liter bottle filled ½ way with deionized water. Mix and dilute to a final volume with deionized water.

- Compressed Air - Recommended Ultra Zero grade

Sample Preparation

Samples may be stored frozen for 28 days in a freezer at or below -10°C. No preparation is needed.

Instrument Analysis

Instrument Start-Up

Turn on the gas, power switch on the back panel of the instrument, and left side of auto sampler. After instrument is on for a few minutes, start computer and open software by clicking <TOC-L sample table editor> at the top of home screen the when the window pops up enter <steph> for used name then <ok>.

Open the front panel of the instrument and make sure the humidifier is filled with DI water between the two marks on the cup.

Check the acid volume and the DI water bottle on the left side of the instrument.

Dump and rinse the 1 gallon amber glass jug to the left of the auto sampler and replace with new DI water.

Click <new> at the top left of the screen. A window will come up that says the instrument Shimadzu TOC-L and Normal Table Type. Press <ok>. Then press <connect> in the top right section of the window. The instrument will initialize.

In the upper right hand corner of the window, press <monitor> and check in over the next hour or so to make sure everything has a green check mark by it. This will indicate the baseline, furnace temperature, gas flow etc. are all stable. When that happens, it is ok to continue running blank samples to stabilize the instrument.

In the sample table highlight <row 1> then <right click> and <insert multiple samples> enter the number <5>.

A window will pop up. Under the method section, click on <…> and click <shimadzu> then <install.met>. Then click on the <…> under the cal curve section then click <12-6-11.cal> and <open> <next>. The cal curve file name is from the first calibration on 12-6-11, it updates this file each time a new calibration is performed.

A new window pops up. For number of samples enter <5> and start vial enter <1>. Change the sample name from “cal” to <rinse> then <finish>.

The vial settings screen pops up and the vial numbers are auto generated. Press <ok>.

Save the file by clicking on the top left of the window <file> <save as> <date in this format 01-29-2020> <save> then insert the rinse vials in the sampler rack and click <start>.

The window pops up asking to save the file. Ensure the date is correctly entered and press <save>.

Another window will pop up asking if, after analysis is complete, the instrument should keep running, shut down or go into sleep mode. If beginning a new run, select <keep running>. If setting up an overnight run, select <sleep mode> and enter 6AM the following morning. This minimized gas usage and wear and tear on the furnace. The following morning the instrument will be ready to run further samples or re-runs.

The instrument holds its calibration for many months before the catalyst must be re-conditioned. If blanks are not stabilizing and the second source quality control sample (QC) doesn't pass within limits, then a new calibration must be performed and/or a new combustion tube must be inserted into the instrument.

Instrument calibration

Create a new schedule as above. Right click on <row 1> and click on <insert-Cal curve> then click on <12-6-11 cal>. The predefined concentrations of 0.000, 1.000, 5.000 and 10.000ppm will be inserted into the spreadsheet.

Use the 1000uL Acculon stock standard to make the 1.0 and 5.0 and 10.0 ppm working standards and record in the standards log book.

Click on the top right corner <sampler icon> and fill in the appropriate vial locations and put the tubes in the auto sampler. Click <start>.

Once the calibration is finished, it will automatically save the file with a new name that is date/time driven. For example, 2-6-11.2020_03_12_16_06_37.cal> was run on 3/12/2020 at 04:06:37pm. Save file as 3-12-2020.

To review a calibration and print the data, click on the tab at the bottom of the sub window in the top left-hand corner of the page that looks like a graph. Pick the correct calibration and review the R^2 value and graphs. The R^2 must be at least 0.995.

Running the First Quality Control

After calibrating the instrument, a second source QC from ERA (cat number 516) is made and run to verify the calibration. It must be within 85-115% recovery to continue with running samples**.** Follow instructions from ERA on making the QC. Prepare fresh daily.

In the same sample table below the calibration line, insert two more samples and add an Initial Calibration Verification (ICV) and an Initial Calibration Blank (ICB) as explained above changing the sample name to ICV and ICB. Click <save>.

Load the auto sampler with the correct samples. Click on <START> to run the sequence.

Creating a Sample Sequence

A 5ppm Continuing Calibration Verification (CCV) is made from the AccuStandard and run every 10 samples. The ERA Initial Calibration Verification is used as a Continuing Calibration Verification (QC) every 20 samples as well. CCV and QC must be made fresh daily. Insert new samples into the sample table as mentioned above.

When running a large group of samples, insert or copy lines from above, deleting the sample ID. Once the samples have been inserted, type the new <sample ID> in the proper column.

The software is similar to excel in that lines can be copied or deleted by right clicking on the line. In this screen it is possible to enter sample ID directly in the column.

Pour of the samples and enter their location in the auto sampler tray correctly on the <vial settings> screen. When finished click <ok>.

To start the run, press <start>. The run will continue throughout the night. A window will pop up asking if, when the run is completed, should the instrument shut down or sleep.

Aborting a Sequence

In the top right-hand corner of the main window, select <stop>. Another window pops up asking if it should stop now or after current sample analysis. Select appropriate condition.

When re-starting after aborting a sample, ensure that a few rinse vials are run to purge any residual sample.

Shutting down the TOC

In the main page, select <shutdown> then <shutdown instrument> <ok> <close>. Wait about a minute until the instrument shuts down and turn off the auto sampler, instrument, and gas tank. Then shut down the computer.

Cleaning the Combustion Tube and Reconditioning the Catalyst

Refer to the manual for cleaning and packing the column.

When a new tube is inserted into the instrument, blank DI water samples should be run until the baseline stabilizes.

Calculations

- Percent recovery for the spike is determined using the following formula:

$$\%R = \frac{(A - B)}{C} \times 100$$

Where:

A = measured value in mg/L for the sample + spike
B = measured value in mg/L for the original sample
C = concentration of the spike in mg/L

- Relative percent difference for the duplicate is calculated by the following formula:

$$\mathrm{RPD} = \frac{(A - B)}{[(A + B) \div 2]} \times 100$$

Where:

A = the value in mg/L for the first run of the sample
B = the value in mg/L for the second run of the sample

Quality Control

- A certified second source quality control sample (purchased from Fisher) is analyzed for every delivery group (or every 10 samples) and the value must be within 85-115% recovery to be considered acceptable.

- A spike is analyzed for every delivery group (or every 10 samples). The percent recovery must fall within the 85-115% recovery to be considered acceptable.

- A duplicate is analyzed for every delivery group (or every 10 samples). The duplicate relative percent difference (RPD) must be below 20%.

- A blank is analyzed every delivery group, or every 10 samples and the value must fall below the PQL to be considered acceptable.

Biochemical Oxygen Demand 405.1 Modified

Scope and Application

EPA Method 405.1 is used in this method. In this method, dissolved oxygen in sea water is measured at 5-day intervals for 30 days. The effect of salinity and temperature on the solubility of oxygen in water is corrected for in this procedure.

Summary of Method

The BOD test is essentially a bioassay procedure involving the measurement of oxygen consumed by living organisms while utilizing the organic matter present in the sample under conditions as similar as possible to those that occur in nature. The sample is placed in an air-tight container and incubated at constant temperature for a pre-selected period of time. In the standard test, a 300mL BOD bottle is used and the sample is incubated at 20°C. for 30 days. Light must be excluded to prevent algae growth, which would produce oxygen in the bottle. Dissolved oxygen is measured initially and every 5 days after incubation. Because of the limited solubility of oxygen in water (about 9 mg/L at 20°C.), the sample is adjusted to approximately 20°C. and aerated with diffused air to increase the dissolved gas content to near saturation at the beginning of the test.

A thin permeable membrane stretched over the sensor of the BOD meter, isolates the sensor elements from the environment, but allows oxygen to enter. When a polarizing voltage is applied across the sensor, oxygen that has passed through the membrane reacts at the cathode, causing a current to flow. If the oxygen pressure increases, more oxygen diffuses through the membrane and more current flows through the sensor. A lower pressure results in loss of current.

Definitions

BOD (Biochemical Oxygen Demand) -- The amount of oxygen required by bacteria while stabilizing decomposable organic matter under aerobic conditions.

Salinity - The electrical conductivity of water relative to a specified solution of KCl and water.

Chlorinity - Salinity * 1000 / 1.8

Nitrifying bacteria - Certain autotrophic bacteria which oxidize, for energy, non-carbonaceous matter such as ammonia which is converted to nitrous and nitric acid. They are usually present in relatively small numbers in untreated domestic wastewater, and their reproductive rate at 20°C is such that their populations do not become sufficiently large to exert an appreciable demand for oxygen.

Inhibiting - the process of adding a chemical such as 2-chloro-6-tri-chloromethyl pyridine to the BOD bottle to eliminate the interference caused by nitrifying bacteria.

Interferences

If large amounts of nitrifying bacteria are present in the sample, errors may be introduced to the BOD results, particularly in the 30-day BOD. Inhibition of nitrification is recommended in these instances. (Not done for LISS seawater samples)

Samples containing residual chlorine may inhibit the reaction. To remove chlorine, aerate for two hours or treat the sample with sodium sulfite. (Not done for LISS seawater samples)

Samples supersaturated with dissolved oxygen (more than 9mg/L) may be encountered in cold water or in water where photosynthesis occurs. To prevent loss of oxygen during incubation of such samples, bring sample to near 20°C and aerate for 15 minutes with compressed air.

Safety

Refer to the University of Connecticut's Environmental Health and Safety Chemical Health and Safety web page at:

http://www.ehs.uconn.edu/ppp/index.php

A hard copy of the Chemical Hygiene Plan can be found on the notebook rack located on the lab bench. Also refer to the Hazardous Waste SOP.

Materials

Dissolved Oxygen Meter—Fisher Accumet XL 40
BOD Bottles - 300mL capacity with ground glass stoppers and plastic caps.
Incubator - Thermostatically controlled at 20°C (+ or - 1°C). All light must be excluded.

Sample Preparation

Samples should be analyzed within 6 hours of collection since samples may degrade significantly during storage between collection and analysis, resulting in low BOD values. If analysis cannot proceed immediately, cool to 4°C in the dark for 48 hours, but no more than 72 hours.

Prior to analysis, samples should be warmed to at least 15°C and aerated with compressed air for a minimum of 15 minutes.

Procedure

Since solubility of oxygen decreases with salinity, this factor must be accounted for by setting the O_2 saturation level to the average LISS salinity of 19ppt.

Label two BOD bottles with sample number and A and C. Bottle C will be used to refill A during the reading intervals. Take readings at 5, 10, 15, 20, 25 and 30 days. If the reading falls below 3.0mg/L at any interval, the sample must be re-aerated. Pour the samples from both bottles into a bottle and aerate with compressed air for a minimum of 15 minutes. Refill the bottles and reread bottle A. Put the initial reading before aeration and the second reading after aeration on the worksheet.

Press and hold the button on the right side of the XL40 meter to turn it on and login with initials and password and press “LOGIN”. Make sure the meter is in DO mode and the correct salinity is in the bottom right box (19ppt for LISS). To change the salinity, press “SETUP” then “CHANGE PPT VALUE” and press “OK”. Press “standardize” on the top right and press “clear” to clear the standardization. Ensure that reading is stable by looking at the graph and press “confirm”. To show the graph press the “graph” button or “hide” to minimize.

Insert probe in sample and turn the red switch on to allow for mixing, and read the BOD. The initial readings should be about 8.0 and should decrease with each progressive reading. Do not leave meter mixing (red switch on) while out of sample!!

Pour off sample on D0, D20 and D30 on designated day in 40mL scintillation vial and add 1 drop of concentrated sulfuric acid. Keep in walk-in cooler.

Cap BOD bottles and keep in BOD incubator in order of LIMS number. Read BOD values every 5 days up until day 30.

To shutdown, press the bottom left corner of the screen and the “**start**” menu will pop up. Press “**shutdown**” then it will prompt if you are sure and press “**yes**”

<u>Calculation</u>:

Day 0 reading minus Day 30 reading = BOD mg/L.

If sample is aerated, this must be accounted for.

Example:

(Day 0 = 8.0 mg/L)
(Day 5 = 3.0 mg/L)
aerated to 8.0 mg/L
(Day 10 = 6.0 mg/L)
(Day 15 = 5.0 mg/L)
(Day 20 = 4.5 mg/L)
(Day 25 = 4.0 mg/L)
(Day 30 = 3.8 mg/L)

Calculation: Day 0 - Day 5 (8.0 - 3.0 = 5.0)
Day 5 aerated - Day 10 (8.0 - 6.0 = 2.0)
Day 10 - Day 15 (6.0 - 5.0 = 1.0)
Day 15 - Day 20 (5.0 - 4.5 = 0.5)
Day 20 - Day 25 (4.5 - 4.0 = 0.5)
Day 25 - Day 30 (4.0 - 3.8 = 0.2)
Total = 9.2 mg/L

Data Review

This document is designed to offer guidance in laboratory data evaluation and validation. In some respects, it is equivalent to a standard operating procedure (SOP) in other, more subjective areas, only general guidance is offered due to the complexities and uniqueness of data relative to specific samples.

Those areas where specific SOP's are possible are primarily areas in which definitive performance requirements are established. These requirements are concerned with specifications that are not sample dependent; they specify performance requirements on matters that should be fully under laboratory control. These specific areas include laboratory preparation and verification blanks, calibration standards, and calibration verifications. The exception to this is the area of individual sample analysis; if the nature of the sample itself limits the attainment of specification, appropriate allowances must be made.

With these concepts in mind, this guideline is designed to permit structured data review. Objective, unambiguous requirements are easily and efficiently delegated to personnel other than experienced professionals. To this end, the guideline is arranged in order, with the most objective, straightforward validation elements given first.

To use this document effectively, the reviewer should have a general overview of the survey at hand. The exact number of samples, their assigned field sheets and laboratory numbers, their matrix, the identity of any field quality control (QC) samples (blanks, duplicates, spikes, splits, performance audit samples), sampling dates and the number of labs involved for their analysis are essential information. Background information and historical data are extremely helpful.

The chain of custody record provides sample descriptions, field stations and corresponding laboratory numbers, and the date of sampling. Although the sampling date is not addressed by contract requirements, the reviewer should be aware of any lag time between sampling and shipping. The case narrative is another source of general information. Notable problems with matrices, insufficient sample for analysis or reanalysis, and unusual events should be found here. The requirements to be checked in validation, in order, are as follows:

I. Sample Holding Times
II. Calibration
    a. Initial Calibration and Calibration Verification
    b. Continuing Calibration Verification
    c. Calibration Blank
III. Blanks
    a. Laboratory Preparation Blank
    b. Field Blank
IV. Specific Sample Results
    a. Duplicate Sample Analysis (Laboratory and Field)
    b. Spike Sample Analysis

Data are reviewed by the analyst and the data report is generated via spreadsheet. The data report undergoes a 100% peer review to ensure that the raw data is in agreement with the reported data. Finally, the report is reviewed by the Laboratory Manager/Senior Analyst or other appropriate personnel for a 30% data review, including checking the agreement of the raw data versus reported results as well as a verification of any calculations. The report then undergoes at 10% review by the Laboratory Director/QA officer. A project narrative is developed for each report and the hard copy as well as the digital copy are delivered to the client.

Sample Holding Times

Objective

The objective is to determine the validity of results based on the holding time of the sample from the time of collection to time of analysis or sample preparation, as appropriate. From the standpoint of the laboratory performance, the time of laboratory receipt until analysis or sample preparation is needed to determine compliance with sample holding time requirements. The holding time is calculated from the receipt of the final batch of samples for each month of collection.

Requirements

The following holding time requirements were established in the Quality Assurance Project Plan for the Long Island Sound Study.

Contract Required Holding Times:

NAME	HOLDING TIME
Ammonia	28 days
Nitrate + Nitrite	28 days
Organic Carbon, Dissolved	28 days
Carbon, Particulate	28 days
Total Dissolved Nitrogen	28 days
Particulate Nitrogen	28 days
Ortho-phosphate	28 days
Total Dissolved Phosphorus	28 days
Particulate Phosphorus	28 days
Dissolved Silica	28 days
Biogenic Silica	28 days
Chlorophyll-a	28 days
Total Suspended Solids	7 days
Biochemical Oxygen Demand	48 hours

Evaluation Procedure

Actual holding times are established by comparing the sample receipt date for the last batch of samples submitted for a particular month on the chain of custody record with the date of analysis found in the laboratory data. Contractual holding times are established by comparing the time of sample receipt with the dates of analysis. Exceeding the holding time for a sample generally affects a loss of the analyte(s). This occurs through any number of mechanisms, such as, depositions on the sample container walls or precipitation. Therefore, when holding time violations occur, the results which are most severely called into question are those which fall close to or below the detection limit. Determination of the effect of holding time violations on the usability of analytical results is extremely subjective. The degree and nature of the effect is dependent on multiple factors, such as the nature of the analyte and matrix, the degree of the violation (days), and the concentration of the analyte in the sample. Ultimately, the decision whether to accept the data is best left to the data reviewer or users professional judgment.

Calibration and QA Acceptance Policy

Initial Calibration and Calibration Verification

Objective

The objective in establishing compliance requirements for satisfactory instrument calibration is to ensure that the instrument is capable of producing acceptable quantitative data. Initial calibration demonstrates that the instrument is capable of acceptable performance at the beginning of the sample analysis runs.

Requirements

For each of the categories listed below the following criteria apply:

- Instruments must be calibrated daily and each time the instrument is setup or as per SOP requirements.

- Calibration verification shall be made by the analysis of certified quality control solutions and materials.

- Where a certified QC sample is not available the accuracy of the calibration shall be conducted on an independent standard at a concentration other than that used for calibration, but within calibration range.

Lachat Auto Analyzer Analysis

- Calibration blank and five standards must be used in establishing the analytical curve. Although this is a rare occasion, it may be necessary to delete a point from the calibration curve. Only one point may be deleted from the curve, and once the point is deleted, the correlation coefficient must yield a value of 0.997 or greater. If a point is deleted, the deleted standard concentration must be re-made and re-run at the end of the run to verify that the standard was made incorrectly or that instrument problems precluded an accurate sample analysis. If the lowest point (the PQL) is deleted from the curve, the next highest standard becomes the PQL and all data between this higher point and the MDL must be flagged.

- Calibration verification must fall within the control limits of 85 to 115% of the true value.

Total Organic Carbon Analysis

- Calibration blank and at least one standard must be used in establishing the analytical curve.

- Calibration verification must fall within the control limits of 85 to 115% of the true value.

Evaluation Procedure

- Verify that the instrument was calibrated at the proper frequency using the correct number of standards and calibration blank.

- Verify that calibration verification source used met contract requirements.

- Review data package for acceptance criteria. Spot check calibration verification for each sample delivery group by recalculation of the percent recovery from the raw data; verify that the recalculation value agrees with the laboratory reported value.

Continuing Calibration Verification (CCV)

Objective

CCV demonstrates that the instrument is capable of acceptable instrument performance (calibration accuracy) over a specific time period.

Requirements

For each of the categories listed below the following criteria apply:

- CCV and continuing calibration blank (CCB) are performed every 10 samples.

- CCV is made by the analysis of certified quality control solutions. Where a certified QC sample is not available the accuracy of the calibration is conducted on an independent standard at a concentration other than that used for calibration, but within calibration range.

- The calibration blank must be less than the contract required detection limit.

Auto Analyzer Analysis

Continuing calibration verification must fall within the control limits of 85 to 115% of the true value.

Total Organic Carbon Analysis

Continuing calibration verification must fall within the control limits of 85 to 115% of the true value.

Evaluation Procedure

Review the supporting raw data to verify that continuing calibration verification and continuing calibration blank analysis were preformed at the proper frequency.

- Verify that calibration verification source used met contract requirements.

- Review data package for acceptance criteria. Spot check calibration verification for each sample delivery group by recalculation of the percent recovery from the raw data; verify that the recalculation value agrees with the laboratory reported value.

Blanks

Objective

The assessment of results on blank analysis is for the purpose of determining the existence and magnitude of contamination problems. The criterion for evaluation of blanks applies to all blanks (reagent, method, and field). The responsibility for action in the case of unsuitable blank results depends on the circumstances and the origin of the blank. If problems with any blank exist, all data associated with the survey and/or sample delivery group must be carefully evaluated.

Requirements

The laboratory preparation blank (reagent blank) is the only in-house blank the laboratory is responsible for reporting and:

- At least one preparation blank must be prepared and analyzed for every sample delivery group (10 samples) received or for each batch of samples digested. Preparation blanks are not analyzed for chlorophyll a, biogenic silica and particulate phosphorus.

- If the concentration of the blank is less than the contract requirement detection limit (CRDL) or project quantitation limit (PQL), no corrective action is required. If the concentration of the blank is above the CRDL or PQL for any group of samples associated with a particular blank, the concentration of the sample with least concentrated analyte must be 10X the blank concentration. The sample value is not to be corrected for the blank value.

- Results must be reported to the instrument detection limit.

- No contractual criteria apply to the levels of contaminant in field blanks.

Evaluation Procedures

Review the results to determine if any blank contamination is identified at levels greater than the CRDL, and then compare blank levels to that of the sample to determine compliance.

Duplicate Sample Analysis

Objective

The relative percent difference (RPD) data is used to evaluate the long term precision of the method for each parameter. The data reviewer can use the results of the duplicate analysis as an indicator of the precision of the sample results.

Requirements

- At least one duplicate sample must be analyzed from each group of samples of a similar matrix or 10 samples, which ever is more frequent.

- Samples identified as field blanks cannot be used for duplicate analysis.

- A control limit of $\pm$ 20% RPD shall be used on sample less than the CRDL or PQL.

- For samples less than the MDL the RPD is not calculated.

- No contractual criteria apply to the results of field duplicates.

Evaluation Procedure

Review data and determine the raw data for the field and laboratory duplicates. Verify the calculation of the RPD and verify results have been correctly reported.

Discussion

Action taken as a result of duplicate sample analysis must be weighed carefully since it may be difficult to determine if poor precision is a result of sample non-homogeneity, method defects or laboratory technique. In general, the results of duplicate sample analysis should be used to support conclusions drawn about the quality of the data rather than a basis of these conclusions.

Spiked Sample Analysis

Objective

The spiked sample analysis is designed to provide information about the effect of the sample matrix on the digestion and measurement methodology. A known quantity of the method analyte is added to an aliquot of sample and analyzed exactly like a sample.

Requirements

- At least one spike sample analysis must be analyzed from each group of samples of a similar matrix or 10 samples, which ever is more frequent.

- Samples identified as field blanks cannot be used for spike sample analysis.

- If the spike recovery is not within the limits of 85 to 115%, the data of all the samples associated with the spiked sample must be flagged to indicate recovery problems. An exception is granted when sample concentration exceeds the spike concentration by a factor of 4 or more or the sample concentration is higher than the highest point on the calibration curve.

- When sample concentration is less than MDL, sample recovery (SR) = 0 may be used for the purpose of calculating recovery if required.

Evaluation Procedure

Review data and determine the raw data for the spike sample analysis. Verify the calculation of the recoveries and verify results have been correctly reported.

Discussion

To properly assess spike sample analysis results, it is necessary for the reviewer to consider a variety of factors which could impact their outcome, such as:

- Matrix suppression effects.

- Matrix enhancement effects.
- Duplicate precision results.
- Digestion efficiency.
- Contamination.
- Relative levels of analyte in spike and sample; for example, if the endogenous sample level is greater than 4 times the spike level the percent recovery results should not be considered accurate or used to judge the accuracy of the sample results.

The following guidelines are recommended for use in evaluating data usability when the spike recoveries do not fall within the required limits:

- If the spike recovery is out of range and the reported sample results are less than the method detection limit (MDL) then this data is acceptable for use.

- If the recovery is out of range and the reported sample levels are greater than the MDL, it should be reported in the project narrative.

- Results of the continuing calibration verification (CCV) should be considered when evaluating spike recoveries. Sample batch acceptance is usually based on results of the CCV rather than the matrix spike alone.

Field Quality Control (QC) and Other Quality Control Techniques

Objective, Definitions and Assessment:

For the LISS, field QC consists of field blanks and field duplicates.

A field blank is DI water that has been "run through" all of the sampling equipment. The intent of a field blank is to monitor for contamination introduced by sampling personnel, although any laboratory introduced contamination will also be present.

A field duplicate is a duplicate water sample collected by the same team or by another sampler or team at the same place, at the same time. It is used to estimate sampling and laboratory analysis precision.

Blanks and duplicates are useful as supporting evidence in the overall assessment of the survey. Blanks are samples of known composition and matrix. As such, they are useful in assessing a laboratory's performance independent of sample or method problems which may arise in a real sample.

Except in the case of gross error, field blanks and duplicates should not be the basis of accepting or rejecting data, but rather additional evidence in support of conclusions arrived at by a review of the total data package. Blanks, spikes, and split sample results will often point out areas the reviewer needs to look at more carefully.